HYGIENIC LABORATORY
BULLETIN No. 154

STUDIES ON ROCKY MOUNTAIN SPOTTED FEVER

U. S. TREASURY DEPARTMENT
PUBLIC HEALTH SERVICE
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TO THE MEMORY OF OUR FELLOW LABORATORY WORKERS WHO, WHILE ENGAGED IN THE STUDY OF ROCKY MOUNTAIN SPOTTED FEVER, HAVE CONTRACTED THE DISEASE AND DIED

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PREFACE

While a practical method of control of Rocky Mountain spotted fever is still to be worked out, the knowledge of the disease in nature and of its behavior in the mammalian and insect host has now reached a stage where it is comparable to that of other insect-borne diseases such as typhus and yellow fever wherein the exact nature of the causative agent is still obscure, and cultivation on artificial media has not been accomplished.

The experimental studies recorded in this bulletin are the result of a close cooperative investigation by the authors over a period of six years—1922 to 1928. The first seven papers have appeared in past issues of the Public Health Reports; the remaining four comprise new observations, and the whole is published under one cover for the sake of convenience and reference.

Grateful acknowledgment is due the members of our field and laboratory staff who in the face of continual risk of their lives, the death of 3 of their comrades, and the illness from either spotted fever or tularæmia of 12 others, have faithfully remained at their post of duty throughout the most trying circumstances.

STUDIES ON ROCKY MOUNTAIN SPOTTED FEVER

INFECTIVITY OF FASTING AND RECENTLY FED TICKS[1]

By R. R. SPENCER, Passed Assistant Surgeon, and R. R. PARKER, Special Expert, United States Public Health Service

During the spring and summer of 1922 certain experiments were conducted at the field laboratory of the United States Public Health Service at Hamilton, Mont., the results of which have added somewhat to our knowledge of the virus of Rocky Mountain spotted fever as it occurs in the tick. Some of these experiments involved the testing for infectivity of large numbers of adult ticks secured in the field; and, since it was believed that the usual method of feeding on guinea pigs was not dependable, a preliminary experiment was carried out in order to compare the results of feeding with the inoculation of macerated tick contents, our criterion of infectivity being either the development of spotted fever or the development of an immunity to spotted fever. Following is an outline of this experiment.

March 16 to 20: Twenty-five adult ticks were fed on infected guinea pig No. 420.

March 22: Twelve of these ticks were selected for testing; 6 were permitted to feed on a guinea pig each for three days; the remaining 6 were dissected and the entire contents of each emulsified in salt solution and inoculated into six pigs, a separate guinea pig being used for each tick.

Results of tick-content inoculation.—Four of the six pigs inoculated with the contents of infected ticks developed spotted fever and two were rendered immune.[2]

Results of tick feeding.—None of the six pigs upon which infected ticks were fed developed spotted fever; one died of pneumonia. After an afebrile period of 10 days the five remaining pigs each received intraperitoneally the contents of the tick which had previously fed upon it. Three of these pigs developed spotted fever as a result of this inoculation; the other two died of an intercurrent infection.

The above experiments, if taken at face value, indicate that the tick-content inoculation method is the more reliable. However, a possible basis for misinterpretation lay in the fact that the ticks used were but recently infected with the virus. Indeed, it was known that many failures have resulted in our own experiences, and in those

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[1] Reprint No. 817 from the Public Health Reports, vol. 38, No. 8, Feb. 23, 1923, pp. 333–339.

[2] Animals are regarded as immune when spotted fever does not develop following an intraperitoneal injection of 1 c. c. of citrated heart's blood of a guinea pig at the height of infection, while it develops in control animals.

of others, in attempting to transmit the virus by the feeding of recently infected adult ticks (infected as adults). Such failures may be due to the existence of an incubation period of unknown duration necessary for the development and distribution of the organism in the tick before it can become infective. (This period has now been demonstrated.) (See p. 52.) Similar experiments are, therefore, being carried out with this possible time element in mind. At the time, however, it was thought that the results of this preliminary experiment were sufficiently suggestive to justify the use of the inoculation method; and although the results of the sum total of our subsequent tests indicate its value, yet it is considered that a combined *feeding* and *inoculation* procedure (Tables 1, 2, and 3) is more dependable in indicating infection. The following observations are our basis for this opinion:

Over 100 lots of drag ticks (drag ticks are unfed males and females secured by dragging a white outing flannel "flag" over vegetation), using 15 to 25 ticks from each lot, were tested by inoculating the tick contents into guinea pigs without once resulting in spotted fever, although many of these tick lots were collected in areas known to be infected. When immunity tests were given to these pigs, many of them were found to be immune.

In view of the results of the preliminary experiment, these results were not easy to interpret until three similarly conducted tests with ticks secured from a mountain goat resulted in spotted fever. The essential difference between the drag ticks and the goat ticks was that the latter had recently ingested blood, whereas the former had not fed since engorging as nymphs not later than during the fall of 1922. The possibility suggested itself that the ingestion of fresh blood was sufficient to reactivate the virus in the tick, since goats are not, as far as known, susceptible to the infection. (Our use of the term "reactivation" is discussed later.)

Our procedure was then changed as follows: Ticks to be tested were first fed on guinea pigs for 48 hours, and if no fever developed within 10 days following the removal of the ticks the contents of the same ticks were then inoculated into the pig upon which they had fed; if fever did not develop in another 10 days the pig was given an immunity test. In order to check the results previously secured by *inoculation alone*, ticks were first tested from some of the same lots of drag ticks which had failed to give spotted fever on immediate inoculation of their contents. These tests included some of those lots which had conferred immunity but failed to give spotted fever. Spotted fever was secured from the first lots thus retested. In some pigs it developed after feeding (Table 1, pig 1100, and Table 2, pig 1095), and in some after the subsequent inoculation of tick contents (Table 3, pig 897); still others were rendered immune, as shown by

the immunity tests (Table 3, pig 819), and others showed neither fever nor immunity (Table 3, pig 891). The following tables are typical illustrations of these results. (Temperatures are in the centigrade scale 39° to 39.6° being normal for guinea pigs.)

TABLE 1.—*Drag tick natural infectivity experiment No. 72*

[Tick lot No. 523]

First test of tick lot Contents of 15 ticks inoculated into pig No. 718, May 6, 1922			Retest of tick lot 25 ticks placed on pig No. 1100, June 16, 1922		
Date	Temperature	Remarks	Date	Temperature	Remarks
May 7	39.2		June 17	39.6	
May 8	39.0		June 18	39.8	Ticks removed.
May 9	39.4		June 19	39.4	
May 10	39.2		June 20	39.2	
May 11	39.2		June 21	39.6	
May 12	39.2		June 22	40.0	
May 13	39.2		June 23	41.0	
May 14	39.2		June 24	41.0	
May 15	39.0		June 25	40.8	
May 16	39.4		June 26	41.0	
May 17	39.6	Immunity test.	June 27	40.4	
May 18	39.6		June 28	40.2	
May 19	39.7		June 29	39.0	
May 20	39.8		June 30	----------	Dead; spotted fever.
May 21	40.0				
May 22	40.4				
May 23	40.6				
May 24	41.0				
May 25	41.0				
May 26	----------	Killed; spotted fever.			

TABLE 2.—*Drag tick natural infectivity experiment No. 61*

[Tick lot No. 326]

First test of lot Contents of 15 ticks inoculated into pig No. 663, May 2, 1922			Retest of lot 13 ticks in capsule fastened to pig No. 1095, June 16, 1922		
Date	Temperature	Remarks	Date	Temperature	Remarks
May 3	39.2		June 17	38.8	
May 4	39.2		June 18	39.6	Ticks removed.
May 5	39.0		June 19	40.0	
May 6	39.2		June 20	39.6	
May 7	39.4		June 21	40.0	
May 8	39.2		June 22	41.0	
May 9	39.8		June 23	40.8	
May 10	39.0		June 24	41.0	
May 11	39.4		June 25	41.0	
May 12	39.4	Immunity test.	June 26	40.6	
May 13	39.0		June 27	40.6	
May 14	38.8		June 28	40.2	
May 15	39.0		June 29	40.0	Killed; spotted fever.
May 16	39.5				
May 17	40.8				
May 18	40.8				
May 19	39.6				
May 20	39.0				
May 21	38.6				
May 22	39.2				
May 23	38.8				
May 24	38.4				
May 25	38.6				
May 26	38.8				
May 27	39.0	Released; immune.			

TABLE 3.—*Examples of results of feeding drag ticks on guinea pigs*

Spotted fever following feeding alone. Pig No. 1095; June 16, 1922. (Repeated from Table 2)			Fever following inoculation of tick contents. Feeding negative. Pig No. 897; May 27, 1922			Feeding and inoculation tests both negative. Ticks infected as shown by immunity test. Pig No. 819; May 17, 1922			Feeding and inoculation negative. No infection of ticks according to immunity test. Pig No. 891; May 27, 1922		
Date	Temperature	Remarks	Date	Temperature	Remarks	Date	Temperature	Remarks	Date	Temperature	Remarks
June 17	38.8		May 28	38.0		May 18	39.4		May 28	38.8	
June 18	39.6	Ticks removed.	May 29	39.0	Ticks removed.	May 19	38.8	Ticks removed.	May 29	38.8	Ticks removed.
June 19	40.0		May 30	39.2		May 20	39.6		May 30	39.2	
June 20	39.6		June 1	39.2		May 21	39.0		May 31	39.0	
June 21	40.0		June 2	39.6		May 22	39.2		June 1	39.0	
June 22	41.0		June 3	39.8		May 23	39.0		June 2	39.0	
June 23	40.8		June 4	39.6		May 24	39.0		June 3	39.0	
June 24	41.0		June 5	39.2		May 25	39.2		June 4	39.0	
June 25	41.0		June 6	39.2		May 26	39.4		June 5	39.4	
June 26	40.6		June 7	39.4		May 27	39.0	Tick contents inoculated.	June 6	38.8	
June 27	40.0		June 8	39.0	Tick contents inoculated.	May 28	39.0		June 7	39.4	
June 28	40.2		June 9	39.2		May 29	39.2		June 8	39.2	Tick contents inoculated.
June 29	40.0	Killed; spotted fever.	June 10	39.0		May 30	38.0		June 9	39.2	
			June 11	39.0		May 31	39.0		June 10	39.4	
			June 12	39.2		June 1	38.8		June 11	39.4	
			June 13	39.6		June 2	38.6		June 12	39.2	
			June 14	40.8		June 3	39.0		June 13	39.4	
			June 15	41.0		June 4	39.0		June 14	39.2	
			June 16	41.0		June 5	38.4	Immunity test.	June 15	39.2	
			June 17	41.0	Spotted fever; typical symptoms.	June 6	38.8		June 16	39.2	
						June 7	39.0		June 17	39.2	
						June 8	38.6		June 18	39.0	
						June 9	39.4		June 19	39.4	Immunity test.
						June 10	39.2		June 20	39.0	
						June 11	39.2		June 21	39.4	
						June 12	39.0		June 22	38.8	
						June 13	39.4		June 23	40.0	
						June 14	39.0		June 24	40.4	
						June 15	39.0		June 25	40.4	
						June 16	38.8		June 26	41.0	
						June 17	39.0		June 27	41.0	
						June 18	39.0	Immune; released.	June 28	38.6	
									June 29	----------	Dead; spotted fever.

Table 3 shows the four results which are possible from testing drag ticks by the combined feeding and inoculation method.

Tables 1 and 2 are typical of the results obtained when drag ticks (adults which had not fed since engorging as nymphs not later than during the fall of 1921) were tested (*a*) by inoculation, (*b*) by feeding and subsequent inoculation if fever did not develop from feeding.

Table 4 gives the results from these two series of tests of drag ticks, (*a*) by immediate inoculation of tick contents and (*b*) by feeding followed by inoculation if no spotted fever resulted from the feeding.

TABLE 4.—*Drag tick infectivity tests*

(*a*) By direct inoculation:	
Total tests completed	101
Tests resulting in spotted fever	0
Tests resulting in immunity	29
Tests negative	72
(*b*) By combined feeding and inoculation (including tests in which the pig sickened with spotted fever after feeding alone, inoculation therefore being omitted):	
Total tests completed	65
Tests resulting in spotted fever after feeding	10
Tests resulting in spotted fever after inoculation, ticks having previously fed	10
Tests showing immunity from feeding and inoculation	8
Tests negative from feeding and inoculation	37

It will be recalled that in the preliminary experiment the inoculation of infected adult tick contents, the ticks having been artificially infected in the laboratory, produced fever, and the *feeding alone* of infected ticks from the same group did not; whereas in the later tests made with drag ticks (naturally infected), the tick content inoculations produced at most immunity in experiments where the feeding of ticks from identical lots produced fever. In interpreting these apparently contradictory results, it must be remembered that in the preliminary experiment the adult ticks ingested the virus only from two to six days before being tested, whereas in the drag tick tests the ticks used were collected in the early spring of 1922 and had not ingested blood since engorging as nymphs not later than the fall of 1921. Under the latter conditions we have not once produced spotted fever by the inoculation of tick contents; but, on the other hand, we have done so repeatedly by permitting them to feed. (Table 3, pig No. 897.)

Mention should also be made of the fact that when testing ticks taken from host animals (as in the case of the mountain goat noted above), the inoculation of the tick contents has frequently resulted in spotted fever, as shown in Table 5. Such ticks had, of course, partially or completely fed within a short time prior to the test.

TABLE 5.—*Animal tick natural infectivity experiment No. 147: Guinea pig No. 1087; inoculated intraperitoneally with contents of 10 D. andersoni nymphs removed from a cottontail rabbit, June 14, 1922*

Date	Temperature	Remarks	Date	Temperature	Remarks
June 15	39.6		June 22	41.2	
June 16	39.2		June 23	41.2	
June 17	39.6		June 24	41.2	
June 18	39.6		June 25	39.6	
June 19	39.6		June 26		Dead; typical spotted-fever lesions.
June 20	41.0				
June 21	41.4				

These results seem to support the supposition upon which we based our idea of a combined *feeding-inoculation* method for testing adult tick infectivity; namely, the ingestion of fresh blood is necessary to "reactivate" the virus in the unfed infected ticks which appear in the spring.

The same tests have been made with the rabbit tick, *Hæmaphysalis leporis palustris* Packard, with similar results. Transmission of the virus by the rabbit tick was demonstrated by one of the writers in 1921. (Parker, 1923a.)

DISCUSSION

1. The above instances do not represent isolated experiments, but are typical of many tests. It is believed that the results indicate that it is unwise to rely upon either *feeding* or *inoculation* alone as an indication of the presence or absence of Rocky Mountain spotted fever virus in unfed adult ticks. The inoculation method alone is apparently most reliable when testing recently fed ticks in all stages.

2. Of particular interest is the nonvirulent immunity-producing phase of the virus that was demonstrated in unfed infected adults (ticks that molted to adults prior to the winter of 1922) when their contents were inoculated into guinea pigs. This phase contrasts strongly with the highly infective phase which frequently developed promptly in such ticks (selected from the same lots) following the ingestion of animal blood. Whenever immunity followed inoculation, it was considered due to the inoculation since, so far as known, no worker in spotted fever has ever found a naturally immune pig. The present writers have inoculated more than 1,000 fresh pigs with virus such as used in the immunity tests, and the results have been uniformly successful.

An immunity producing phase also occurs in the eggs as first shown by Ricketts. (Ricketts, 1907a.) Spotted fever was produced by the injection of eggs of infected females, using from 5 to 80 eggs. These eggs, however, were less than a week old. With eggs that had been dried for four months, immunity instead of fever developed.

In an attempt to cultivate the virus, Fricks (Fricks 1916–a) in 1915 inoculated infected tick eggs which had been incubated with a special culture medium for 25 days and produced immunity in the inoculated pig.

In 1921, one of the writers (Parker) produced immunity by the inoculation of comparatively fresh eggs from an infected rabbit tick, the immunity test being given three months after inoculation.

3. The term " reactivation " is used for lack of a better descriptive word to designate the transition of the virus from a nonvirulent immunity-producing phase to a virulent fever-producing phase. It is not known whether this transition is due to multiplication of the virus, to development of a possible distinct stage in its life cycle, to renewal of virulence following a period of attenuation, or, perhaps to some other unrecognized condition initiated by the ingestion of fresh mammalian blood.

This phenomenon provides an explanation of the fact first demonstrated by Ricketts, that ticks usually do not infect unless attached for some hours. In a single instance he secured infection in one hour and three-fourths, but found that 10 to 20 hours' feeding was usually required. In our studies we have repeatedly fed ticks for 48 hours without securing infection; whereas the presence of the virus was afterwards demonstrated, either by infection following inoculation of the contents of the ticks concerned or by the subsequent immunity test. (Table 3, pigs No. 897 and 819.) It seems probable, therefore, that when unfed infected adult ticks have hibernated, the virus must be reactivated by fresh blood before such ticks become infective. Furthermore, it is possible that this apparent need of blood furnishes an explanation of the comparatively small number of human cases which occur. Many ticks which attach themselves to human beings are doubtless removed before " reactivation " has taken place.

The study of this phenomenon suggests the possibility that the virus may sometimes die out in the tick. Observations made by King [3] have shown that unfed adult ticks frequently live for two and three years and occasionally into the fourth season. If the ingestion of fresh blood is essential for the " reactivation " of the virus, it seems probable that in instances when infected ticks do not secure hosts for extended periods of time the virus may die or its vitality be so reduced that " reactivation " can not occur. The results shown by Table 3 may be indicative of different periods of fasting on the part of ticks infected in nature; although immunity which could not be ascribed to such cause has occasionally been unexpectedly produced.

[3] King, W. V.: Unpublished experiments.

VIABILITY OF THE VIRUS IN ANIMAL TISSUES [4]

By R. R. SPENCER, Surgeon, and R. R. PARKER, Special Expert, United States Public Health Service

Ricketts (Ricketts, 1907c) found that complete desiccation of the virus of Rocky Mountain spotted fever in blood would destroy pathogenicity in 24 to 48 hours and that virus kept in the ice chest retained its infectiousness for 16 days, although the minimal infectious dose greatly increased.

Wolbach (Wolbach, 1919b) found that complete desiccation destroyed the virus in blood in 10 to 15 hours. It withstood freezing longer than four days and less than nine days. He found the virus in testes, liver, spleen, and kidney infectious after it had been in 25 per cent and 50 per cent glycerin at 7° to 10° C. for five days, but it was destroyed after one month. Intermediate periods were not tested.

That the virus survives in the tissues of the fever tick (*Dermacentor andersoni*) through extremely cold weather may be inferred from the feeding habits and life cycle of the tick. Recently this has been demonstrated experimentally by keeping infected ticks outdoors through the winter in the Bitter Root Valley of Montana. In the spring of the following year the presence of the virus in infective quantities was shown, either by incubating the ticks at 37° C. for 24 hours and then inoculating their contents intraperitoneally into guinea pigs (see p. 12) or by permitting the ticks to feed upon guinea pigs for two days. Inoculation with infected, unfed, wintered ticks without incubating or feeding has never produced a frank infection, as shown in a previous paper. (See p. 2.)

With this in mind, it was believed that the virus, under suitable conditions, would also survive in mammalian tissues. Therefore tissues were removed from guinea pigs at the height of typical spotted-fever symptoms and when showing no evidence of secondary infection. Such tissues were treated as shown in the accompanying tables, and tests for viability of the virus were made at intervals by emulsifying from one-half to 1 gram of tissue in 1 to 2 c. c. of salt solution and inoculating guinea pigs intraperitoneally. The time of survival of the virus varied greatly, and the tables given below represent selected tests.

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[4] From the Public Health Reports, vol. 39, No. 2, Jan. 11, 1924, pp. 55–57.

TABLE 6.—*Survival of spotted-fever virus in tissues of guinea pigs*

[Guinea pig No. 6527. Tissue removed from guinea pig October 16, 1922]

Test No.	Tissue	Treatment of tissue	Date of inoculation	Result
1	Spleen and liver	100 per cent glycerin kept at about —10° C.	Nov. 20, 1922	Typical spotted fever, with gangrene of testicles. Heart blood culture negative. Virus survived 35 days.
2	do	do	do	Typical spotted fever. Heart blood transferred to 3 pigs, all of which developed spotted fever. Virus survived 35 days.
3	do	do	do	Typical spotted fever. Virus survived 35 days.

TABLE 7

[Guinea pig No. 6520. Tissue removed from guinea pig October 30, 1922]

1	Testicle	100 per cent glycerin and — 10° C.	Dec. 30, 1922	Typical spotted fever. Virus survived 2 months.
2	Spleen	do	do	Do.
3	do	do	do	Do.

TABLE 8

[Guinea pig No. 7585. Tissue removed from guinea pig November 16, 1922]

1	Spleen	Spleen chopped into small bits, chilled in CO_2 snow, and placed in vacuum H_2SO_4 at —10° C.	Jan. 16, 1923 [1]	Typical spotted fever. Virus survived 2 months.
2	do	do	do	Do.
3	Liver	do	do	Do.
4	do	do	do	Do.

[1] Vacuum good on removal.

TABLE 9

[Guinea pig No. 6374. Tissue removed from guinea pig January 2, 1923]

1	Testicle	100 per cent glycerin kept at about —10° C.	Nov. 14, 1923	Typical spotted fever. Virus survived 10 months and 12 days.
2	Spleen	do	do	Negative—spotted fever following injection of fresh virus.

TABLE 10

[Guinea pig No. 6131. Tissue removed from guinea pig January 4, 1923]

1	Testicle	100 per cent glycerin kept at about —10° C.	Nov. 14, 1923	Typical spotted fever. Heart blood culture sterile 48 hours on agar slant and anaërobic meat media. Survival of virus 10 months and 10 days.
2	Spleen	do	do	Negative—spotted fever following injection of fresh virus.

TABLE 11

[Guinea pig No. 7117. Tissue removed from guinea pig January 12, 1923]

1	Testicle	100 per cent glycerin kept at about —10° C.	Nov. 3, 1923	Typical spotted fever. Surviva over 9 months.
2	Liver	do	do	Negative. Dead on eighth day from secondary infection.

The above tables indicate that--

1. The virus of Rocky Mountain spotted fever in guinea-pig tissues survives in glycerine for more than 10 months when kept at −10° C.

2. The testicle is a more favorable tissue for the preservation of the virus in glycerine than is the spleen or liver. Brain tissue was not tested.

3. Tissue virus dried in vacuo survived two months when kept at −10° C.

No loss of virulence of the virus kept in glycerine was noted, since the eighth passage of the virus still showed full virulence for guinea pigs, producing typical lesions.

EXPERIMENTAL STUDIES ON TICK VIRUS[5]

By R. R. Spencer, Surgeon, and R. R. Parker, Special Expert, United States Public Health Service

The virus of Rocky Mountain spotted fever may be studied as it occurs in the tissues of susceptible mammals (tissue virus) or in the intermediate hosts, *Dermacentor andersoni* Stiles and *Haemaphysalis leporis-palustris* Packard (tick virus). Observations during the past two years have indicated that tick virus possesses interesting and perhaps significant phases in its development not observed in tissue virus. In a previous paper (see p. 2) we described a nonvirulent immunity-producing phase of the tick virus in unfed infected ticks, and a reactivation of such virus to a virulent infectious stage following the ingestion of fresh blood by the ticks. These preliminary studies were made on infected ticks collected in nature. For further and more detailed observations, ticks have been infected under controlled conditions. To this end the progeny of single noninfected females have been used as units designated by lot numbers and have been infected by permitting them to engorge during either the larval or nymphal stage upon a rodent inoculated with spotted fever virus. In this way many infected ticks of identical history have been secured. Furthermore, this method of infecting ticks is comparable in large measure to that taking place in nature, since the immature stages[6] feed on wild rodents susceptible to spotted fever.

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[5] Reprint No. 976 from the Public Health Reports, Vol. 39, No. 48, Nov. 28, 1924, pp. 3027–3040.

[6] *D. andersoni,* like many other external parasites, undergoes an interesting and complicated life cycle. The adult female, after engorging to many times its normal size, drops from its host and crawls to a sheltered place. Before leaving the host the female is impregnated by the male, which feeds only a short time before seeking its mate. The female remains quiescent a week or more, depending on the temperature, and then begins the deposition of eggs—from 2,000 to 7,000 in number. This sometimes takes a month or even longer. These hatch to seed ticks or larvæ, which are not more than one thirty-second part of an inch in their longest diameter. These 6-legged larvæ feed on rodents, such as ground squirrels, chipmunks, field mice, rabbits, etc.; 50 or more may be found on one small host. After feeding from two to four days and attaining the size of a millet seed, they drop to the ground, pass through a dormant stage, and shed the outer skin, emerging as 8-legged nymphs which are sexually undifferentiated. They do not

On the other hand, infection is seldom acquired by adult ticks, the majority of which feed on nonsusceptible large mammals, wild and domestic.

In previous experimental work the importance of infecting ticks during one of the immature stages has not been emphasized. While adult ticks are more readily obtained and more easily handled and controlled than the smaller nymphs and larvæ, nevertheless, infected at the adult stage, they often fail to transmit the fever when permitted to feed again upon a healthy animal, although the injection of their viscera soon after feeding is usually successful.[7] However, adult females receiving the infection and permitted to come to full engorgement may transmit it through the eggs to the next generation. The infection may then be recovered in animals by (*a*) injection of deposited eggs—a single egg (0.0006 gm.) has been found infective—(*b*) feeding the resultant larvæ or nymphs, (*c*) injection of the larval or nymphal contents. The last test should preferably be made soon after molting and before hibernation of the larvæ or nymphs.

On the other hand, adults which have been infected during one of the immature stages of the life cycle, if tested after passing through the winter, readily infect animals by feeding, whereas injection of tick contents does not infect unless the ticks are first incubated or fed. Further, the virus in fed adults infected in either of the early stages has been found to be more highly fatal and more concentrated than either tissue virus or tick virus at other stages. Recent tests, not included in this paper, indicate considerable concentration of virus in engorged nymphs which have been infected as larvæ and in engorged larvæ which have been infected in the previous generation. In addition, the killed virus of such adult ticks possesses an immunizing quality never encountered in blood virus and only rarely in animal tissue virus.

Before giving our data in support of these statements certain general considerations relative to the underlying conditions in nature and upon which the maintenance of Rocky Mountain spotted fever

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become active, however, until the following spring. Like the larvæ, they feed on rodents and engorge in from 3 to 10 days, finally reaching a size slightly smaller than buckshot. The engorged nymphs then molt to the adult ticks—males and females—which pass the winter in a dormant condition. The adults attach themselves only to large animals, including man, and are seldom found on animals smaller than a jack rabbit. The larval and nymphal ticks, on the other hand, have never been found on any but small animals, though occasionally nymphs have been removed from children. Although under normal conditions the cycle from egg to adult is completed in two years, it frequently happens that the ticks do not secure a host during the season in which they become active. This causes a high mortality among the larvæ and nymphs. The adults are able to survive for two, three, and occasionally four years without feeding. In this way the life cycle may be considerably lengthened. In the laboratory, however, they are often forced by artificial means to complete the cycle in three months.

(The life cycle of *Haemaphysalis leporis-palustris* (rabbit tick) has not been completely worked out for this locality (Hamilton, Mont.).)

[7] Spencer, R. R., and Parker, R. R.: Loc. cit.

rests should be briefly reviewed. Chief among such considerations are—

1. The disease is maintained in rodents and ticks; human cases are secondary and accidental.

2. The disease exists in definite foci, and the virulence of the infection may vary decidedly even in adjacent areas.

3. A large number of rodent species are susceptible, but there is no evidence that the infection is highly fatal among them.

4. The complete life cycle of the tick includes four stages, and the infection may pass from stage to stage and from one generation to the next.

5. A disintegration of tissue (histolysis) takes place during the premolting period of larvæ and nymphs.

6. The tick ingests mammalian blood three times during the cycle—twice from small rodents (most of them susceptible to spotted fever) as larvæ and nymphs and once from large animals (all immune as far as known except that some adults feed on jack rabbits, snowshoe rabbits, and porcupines, of which animals at least the first two are susceptible to spotted fever) as adults.

7. The virus passes through the egg and larval stages of the tick in one summer, but in the unfed nymphs and adults it has been compelled to adapt itself to the hibernation (also æstivation in the adult) which these stages undergo.

8. The infective agent experiences a sudden change in environment when it passes from mammalian blood to that of the insect host, and vice versa.

9. The mammalian host provides a far more regular and unchanging environment as regards temperature for the virus than the cold-blooded tick.

1. DEVELOPMENTAL PHASES OF TICK VIRUS

In Table 12 it may be seen that infected adult ticks, infected as larvæ, lot 2351–B,[8] recently molted and not subjected to cold, produced spotted fever when injected intraperitoneally into guinea

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[8] History of lot 2351–B—

Apr. 11, 1923: Only fully engorged female tick secured from a horse west of Hamilton, Mont.

May 5, 1923: Eggs deposited by female hatched to larvæ.

July 1, 1923: Larvæ placed on a Belgian rabbit which had previously been innoculated with 1 c. c. of *guinea pig virus*.

July 9, 1923: Twenty-five fully engorged larvæ injected into guinea pig No. 3986. Typical spotted fever developed.

Aug. 8, 1923: Engorged larvæ had now molted to flat nymphs, and the latter were placed on a normal Belgian rabbit.

Aug. 15, 1923: Five engorged nymphs injected into guinea pig No. 4637. Animal developed typical spotted fever.

Sept. 8, 1923: Engorged nymphs had all molted to adults, some of which were forwarded to the Hygienic Laboratory at Washington and placed in the ice box (0° C.), while the others were placed outdoors in glass cylinders at Hamilton, Mont.

pigs. Ticks from the same lot after 39 days, and again after 112 days, in the ice box did not produce fever upon injection. However, 4 out of 8 ticks so tested immunized the guinea pigs against 1 c. c. of blood virus given 10 days after the injection. Yet simultaneous tests with ticks from the same lot incubated at 37° C. for 24 hours after removal from the ice box produced spotted fever in 6 out of 8 guinea pigs.

TABLE 12.—*Studies of tick virus in adults of lot No. 2351–B–(2A)—Intraperitoneal injection of tick viscera into guinea pigs, contents of one tick into each pig—All pigs surviving tick inoculation 10 days or more were given 1 c. c. of blood virus*

Test No.	Date	Condition or preparation of tick	Result of guinea-pig inoculation
1	Sept. 12, 1923	Recently molted, kept at room temperature	Typical spotted fever.
2	do	do	Do.
3	do	do	Do.
4	Oct. 31, 1923	Ice box 39 days, Sept. 12 to Oct. 31	No fever.
5	do	do	Do.
6	do	do	Do.
7	do	do	No fever; later immune.
8	Nov. 1, 1923	Ice box 39 days; 37° C. for 24 hours	Typical spotted fever.
9	do	do	Do.
10	do	do	Do.
11	do	do	No fever; later immune.
12	Jan. 2, 1924	Ice box 112 days, Sept. 12, 1923, to Jan. 2, 1924	No fever.
13	do	do	No fever; later immune.
14	do	do	Do.
15	do	do	Do.
16	Jan. 3, 1924	Ice box 112 days; 37° C. for 24 hours	No fever.
17	do	do	Typical spotted fever.
18	do	do	Do.
19	do	do	Do.

TABLE 13.—*Studies of tick virus in unfed nymphs of lots 969c and 788—Intraperitoneal injection of tick viscera into guinea pigs, contents of one tick into each pig—All pigs surviving tick inoculation 10 days or more were given 1 c. c. of blood virus*

Lot data	Not incubated		Incubated	
	Taken from ice box Dec. 17, 1922, and inoculated immediately		Taken from ice box Dec. 19, 1922, incubated 24 hours at 37° C., and then inoculated	
	Tick No.	Result of inoculation	Tick No.	Result of inoculation
Lot 969c Larvæ infected Aug. 31, 1922. Began molting to nymphs Sept. 18, 1922. Unfed nymphs kept in ice box.	1	Spotted fever, fatal.	19	Spotted fever, fatal.
	2	Spotted fever, recovered.	20	Do.
	3	Do.	21	Do.
	4	Do.	22	Do.
	5	Immunity.	23	Do.
	6	Negative.	24	Do.
	7	Do.	25	Do.
	8	Do.	26	Negative.
	9	Do.		
	10	Do.		
	Taken from ice box Dec. 19, 1922, and inoculated immediately		Taken from ice box Dec. 20, 1922, incubated 24 hours at 37° C., and then inoculated	
Lot 788 Larvæ infected Aug. 2, 1922. Began molting to nymphs Aug. 18, 1922. Unfed nymphs kept in ice box.	11	Immunity.	27	Spotted fever, fatal.
	12	Do.	28	Do.
	13	Do.	29	Do.
	14	Do.	30	Do.
	15	Negative.	31	Do.
	16	Do.	32	Do.
	17	Do.	33	Spotted fever, recovered.
	18	Do.	34	Do.
			35	Do.
			36	Do.
			37	Negative.

Table 13 also demonstrates the increased virulence of tick virus following incubation, in this case in unfed infected nymphs. It will be noted that some of the nonincubated nymphs of lot 969–C produced mild spotted fever, whereas when infection occurred due to inoculation with the incubated nymphs it was always fatal. Although mild infection followed injection of several of the nonincu-

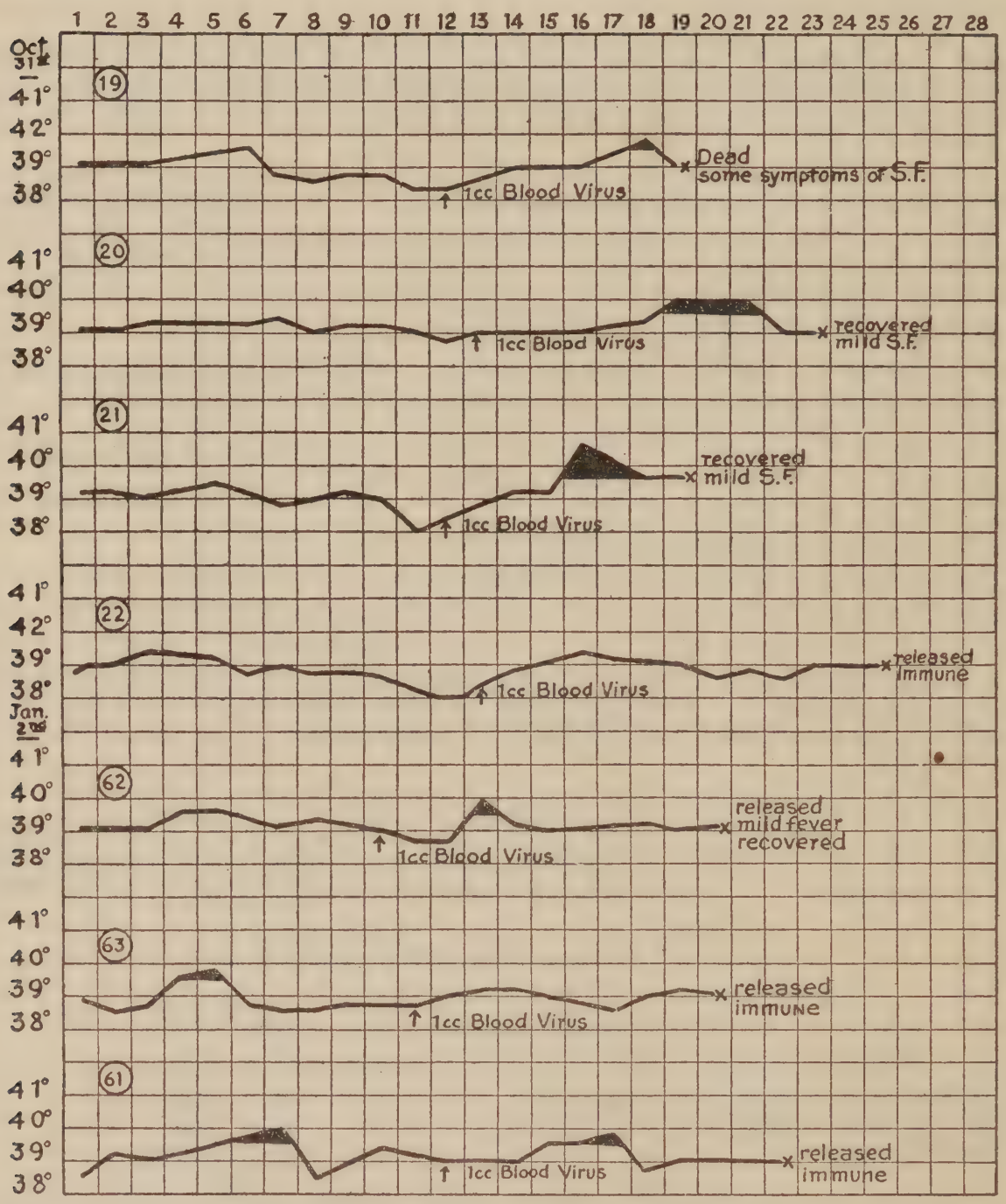

FIGURE 1.—Lot 2351–B–(2A). Unincubated and unfed ticks injected immediately upon removal from ice box

bated nymphs of the above lot, the nonincubated nymphs of lot 788 produced immunity only, closely paralleling results with nonincubated infected adults.

Figures 1, 2, and 3 have been prepared to compare the virulence of spotted-fever virus in unfed, unfed and incubated, and incubated and fed adult ticks.

Figure 1 gives the temperature curves of guinea pigs injected with one infected tick each, taken directly from the ice box. The first four tests were carried out on October 31, 1923, the last three on January 2, 1924. In five pigs no fever followed the injection. Two showed an elevation of 39.8° C. and 40° C., respectively, for one day

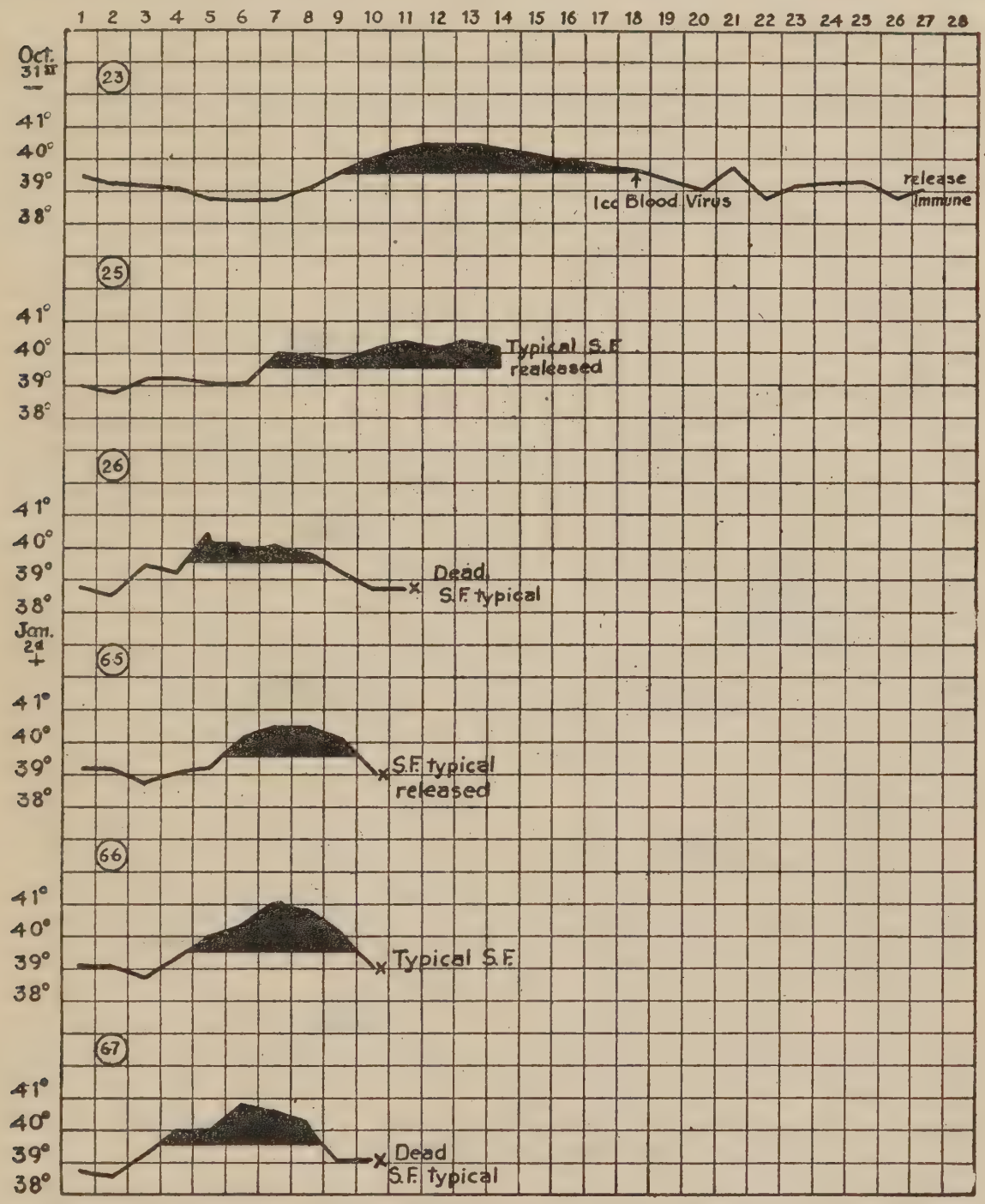

FIGURE 2.—Lot 2351–B–(2A). Unfed ticks removed from ice box, incubated 24 hours at 37° C., and injected peritoneally

each.[9] The subsequent injection of blood virus was negative in two pigs, indicating complete immunity. The others developed mild

[9] We have considered any temperature in guinea pigs above 39.6° C. to be a definite fever, and areas in the chart lying between this line and the temperature curve are shaded in black. While some investigators consider 39.2° C. to be the upper limit of a normal guinea pig's temperature, it is believed the temperature varies considerably with that of the surrounding air and the age of the animal. Young pigs run a consistently higher temperature than those which have matured

fevers. The results here are similar to those obtained when unfed, hibernated adults from nature are tested by injection into guinea pigs.[10]

The tests shown in Figure 2 were identical with those shown in Figure 1, except that in the former the ticks were incubated 24 hours at 37° C. All the guinea pigs developed spotted fever with typical external lesions and survived 10 days or more.

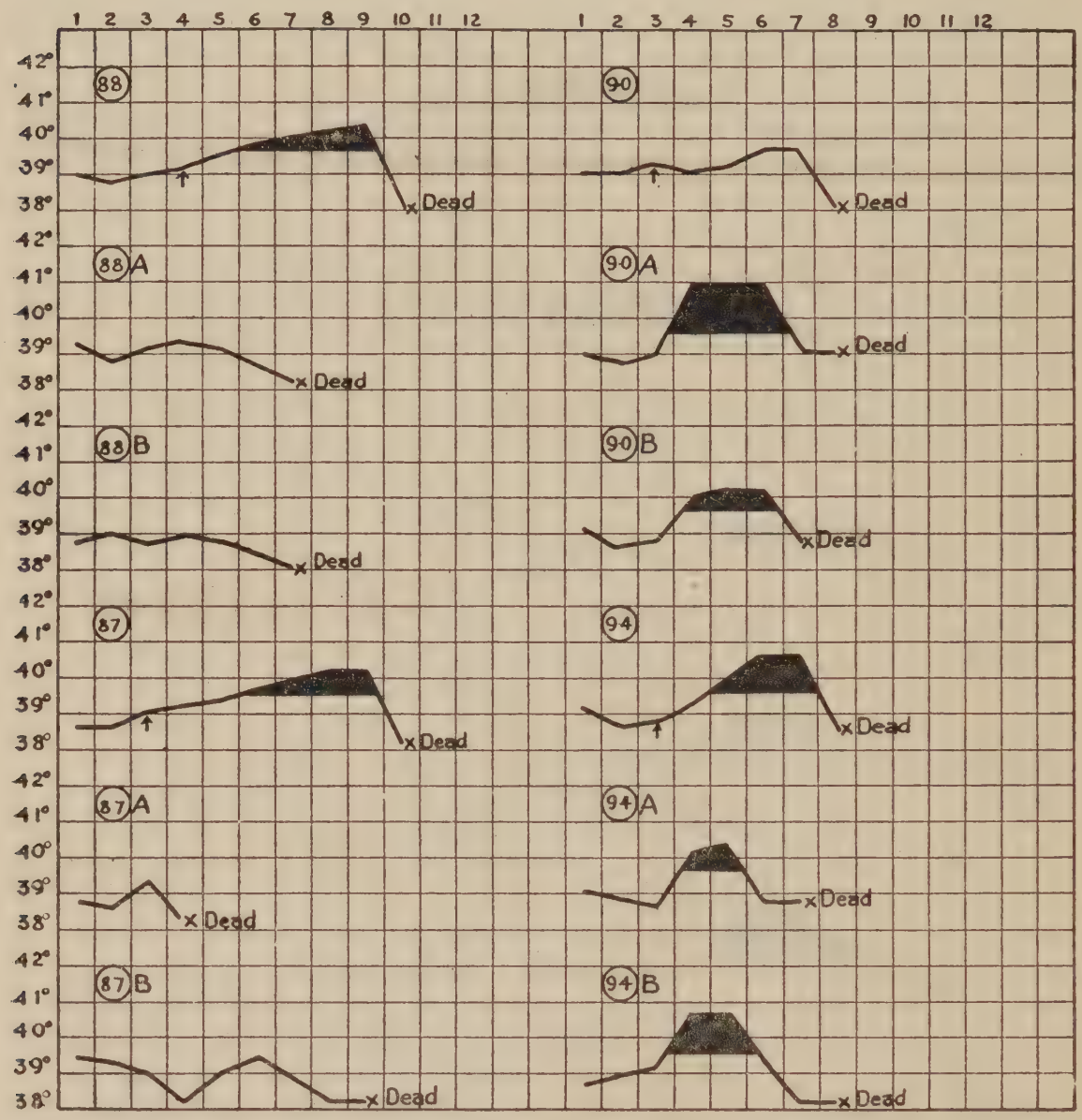

FIGURE 3.—Lot 2351–B–(2A). Ticks removed from ice box, incubated 24 hours at 37° C., then fed three days and injected intraperitoneally

Figure 3 shows temperature curves of guinea pigs Nos. 87, 88, 90, and 94, upon each of which two ticks fed for three days (the arrows indicate the day on which the ticks were removed), and those of guinea pigs 87–A and 87–B, 88–A and 88–B, 90–A and 90–B, 94–A and 94–B, which were injected with the viscera of the ticks after removal. All 12 guinea pigs died, none surviving the tenth day, the majority dying within eight days, and one as early as four

[10] Spencer, R. R., and Parker, R. R.; Loc. cit.

days. Post-mortem examination revealed the lesions of spotted fever in all cases, although five of the pigs ran practically an afebrile course, the temperature never exceeding 39.6° C. In the case of pig 87–A, which died on the fourth day without elevation of temperature, two fresh pigs were inoculated intraperitoneally with an emulsion of the spleen. Both animals ran a fever and showed scrotal lesions of spotted fever.

Figure 4 presents the temperature curves of three guinea pigs upon which there fed, respectively, 10, 13, and 9 ticks from the uninfected control lot 1988–E. After three days' feeding they were removed and their emulsified viscera injected intraperitoneally into their respective hosts. No elevation of temperature followed. After 12

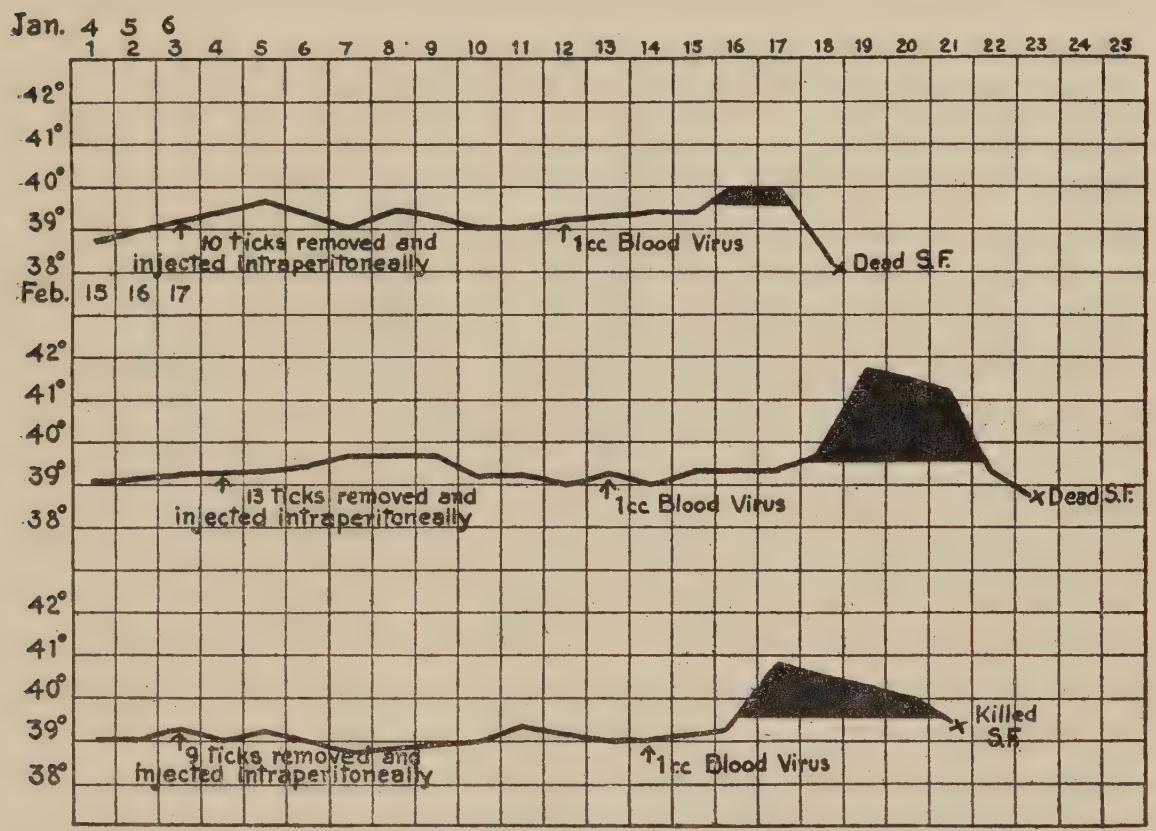

FIGURE 4.—Lot 1988–E. Noninfected control ticks

days 1 c. c. of blood virus was given and was followed by typical spotted fever. This chart shows that a large number of uninfected fed ticks will not kill guinea pigs either by feeding or intraperitoneal injection of their viscera after feeding, nor will such injections immunize.

Comparative studies of Figures 1, 2, and 3 reveal remarkable differences. Of 7 guinea pigs inoculated with *unfed, unincubated infected* ticks (fig. 1), not one developed typical spotted fever, although the presence of the virus was indicated by the results of subsequent immunity tests, some guinea pigs being entirely and

[10] Spencer, R. R., and Parker, R. R.; Loc. cit.

some partly immune. Of 6 guinea pigs injected with *incubated infected* ticks (fig. 2), all developed spotted fever, but survived 10 days or longer. Of 4 guinea pigs on which 2 *incubated infected* ticks each were allowed to feed (fig. 3), all died of spotted fever in 10 days or less, and of 8 guinea pigs into which these identical *fed* ticks were inoculated, all died in 4 to 9 days. These differences are observed in a lot of ticks, the progeny of a single female. They were infected on the same host at the same time and subsequently kept in the same environment until the beginning of the test. Virulence of low grade is manifested in *unfed*, *unincubated* ticks taken directly from the ice box; virulence sufficient to produce definite spotted fever but no early deaths is seen in the *unfed*, *incubated* ticks, and a virulence of high degree in ticks *incubated* 24 hours at 37° C. and then *fed* for three days on a guinea pig. In brief, a progressive development of the virulence has taken place. Nothing comparable to this is seen in a strain of tissue virus, the virulence of which remains fairly constant for months.

Many more tests aside from those given in Figures 1, 2, and 3 were performed. Some ticks of this infected group (2351–B) failed to give infection, but no results were obtained inconsistent with those outlined in the charts. Figure 4, however, represents the total number (32) of uninfected ticks tested as controls.

Figure 5 represents the results of injecting guinea pigs on January 5, 1924, with various dilutions of a tick virus emulsion. The viscera of infected adults of lot 2351–B were ground in a mortar with a small amount of salt solution. Dilutions were made so that 1 c. c. of each represented a definite fractional part of a tick and each such fraction was inoculated into two guinea pigs. All guinea pigs receiving from 1/3 to 1/3000 of a tick developed spotted fever, except one animal that received the latter dilution. Although this guinea pig developed fever, it was shown not to be spotted fever by the subsequent immunity test. Both 1/30000 dilutions were negative. Subsequent titrations of virus from the same tick lot made on March 28, 1924, gave an infectious dose as low as 1/5000 of a tick.

The contents of one adult tick after three days' feeding (the same as used for titrations) weighs about 0.01 gram and therefore 5,000 M. I. D. (minimum infectious dose) of tick virus may be concentrated in this amount of recently fed tick tissue. The tick has served, therefore, as a more efficient culture media than the guinea pig, the blood of which is infectious in minimal doses of from 1/100 to 1/1000 c. c. On this basis, tick virus of adult ticks when reactivated by freshly ingested blood may contain, volume for volume, five hundred to five thousand times as many M. I. D. as guinea pig serum virus.

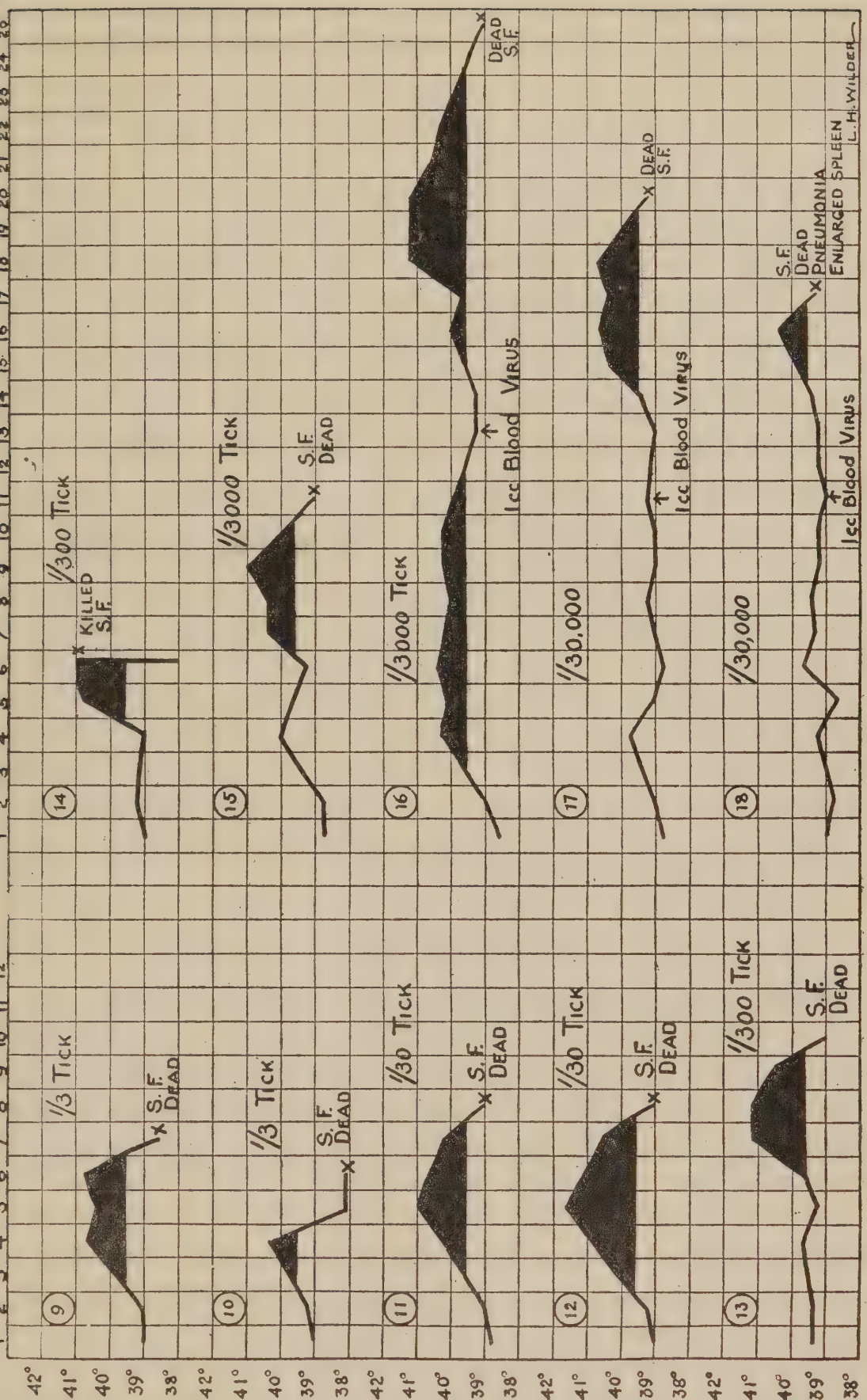

FIGURE 5.—Titration of virus in fed ticks

The difficulties experienced in rearing rabbit ticks (*Haemaphysalis leporis-palustris* Packard) have thus far prevented titration of the virus of this species, which is, perhaps, as equally important a factor in the maintenance of spotted fever in nature as *Dermacentor andersoni.*

On the same date (March 28, 1924) that titrations of lot 2351–B tick virus gave 1/5000 of a *fed* adult tick as the M. I. D., control tests with *unfed* adults of the same lot were made in order to exclude a spontaneous increase in virulence and infectiousness in the unfed ticks during their long exposure to a cold environment without the stimulus of heat or blood (subsequent to the tests made on October 31 and January 2, see fig. 1). Eight *unfed* and *unincubated* ticks of this lot kept outdoors at Hamilton, Mont., all winter were injected into eight guinea pigs on March 28, 1924. None of these died or developed a typical spotted fever, but three were immune to a subsequent injection of blood virus. This result was similar to that secured by a similar test on four of these ticks on October 31 of the previous year (fig. 1), and, therefore, indicate that the results of the titration as made on January 5 (fig. 5) and March 28, 1924, were due to a reactivation of the tick virus by *incubation* and *feeding* (titration of January 5, fig. 5) and feeding alone (March 28) in the respective instances. It should be stated that on January 5 the ticks would not feed without incubation, whereas on March 28 incubation was not necessary. Feeding alone is apparently sufficient to bring the virus to its highest virulence and concentration, and it is necessary to resort to previous incubation only during the winter months, when ticks usually refuse to feed.

2. INJECTION OF PHENOLIZED TICK VIRUS

Figure 6 gives the temperature records of 10 guinea pigs inoculated subcutaneously with 1 c. c. of infected, fed, adult tick viscera emulsified in salt solution, the virus having been killed by the addition of 0.5 per cent phenol. The " vaccine " was prepared so that each cubic centimeter contained the equivalent of one tick. Before being used it had remained in the ice box over 20 days. Titration of the virus before the addition of phenol gave a M. I. D. of 1/5000 tick. Therefore each animal received 5,000 infectious doses of killed virus. Guinea pigs Nos. 5615 and 5616 were control animals which demonstrated the infectiousness of the blood virus given on the fourteenth day after inoculation. Guinea pig No. 5495 gave a fever of short duration on the eleventh day following the immunity test, but there was no evidence of spotted fever. Only two guinea pigs, Nos. 5498 and 5500, had elevation of temperature following vaccination. Their temperatures each reached 40° C. for one day but were probably not due to the vaccine.

The table indicates that the killed virus in the contents of one fed, infected tick is sufficient to protect guinea pigs. Vaccine preparations from other infected tick lots have never failed to protect when the same amounts are used, and therefore further concentration of the material has not thus far been attempted. The duration of this immunity, the minimal immunizing dose, and the period following vac-

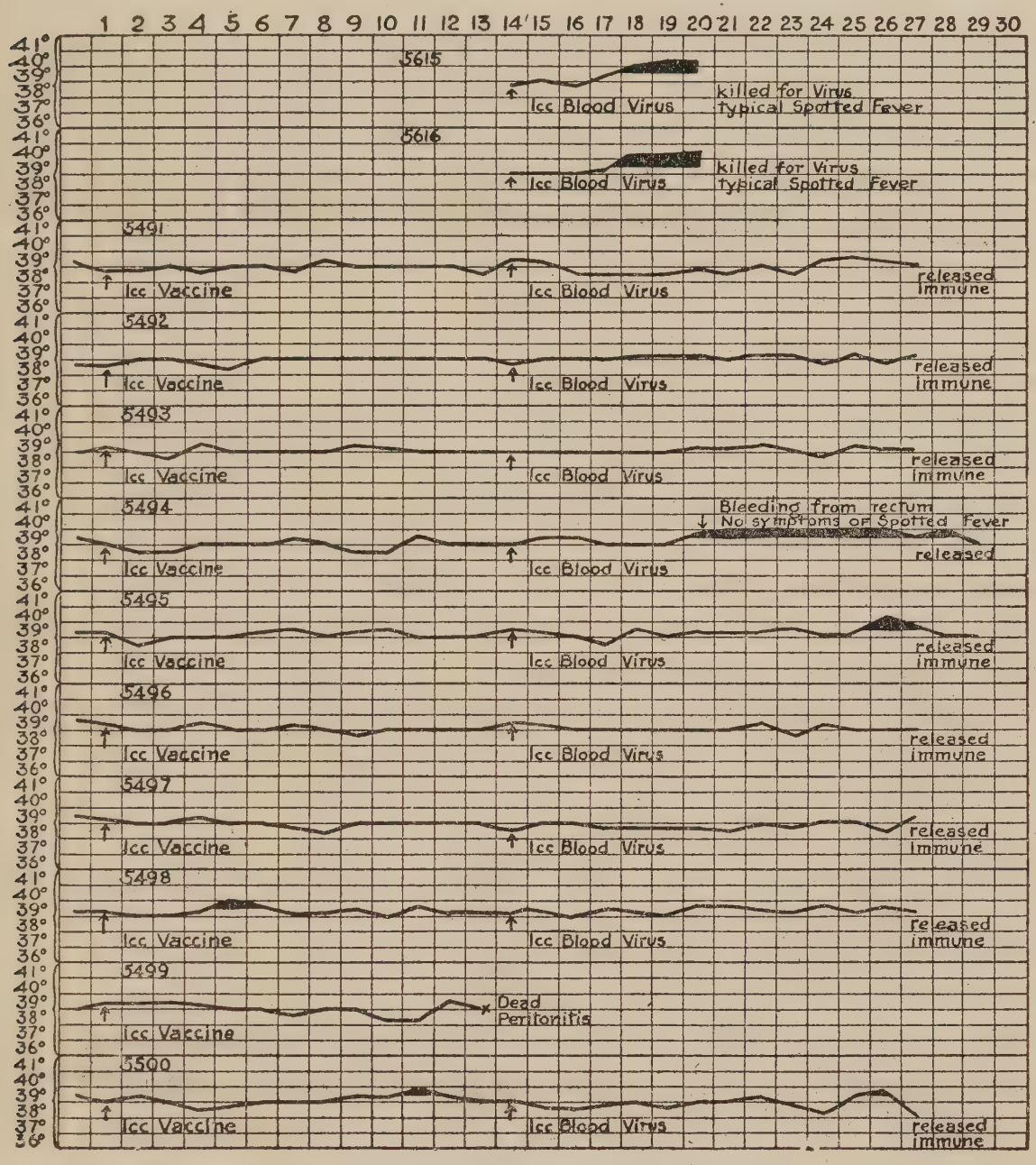

FIGURE 6.—Injection of phenolized tick virus

cination before immunity is established are not yet known. (See later results, p. 70.) The feasibility of human vaccination also naturally arises. In this connection, the relative harmlessness of tick material is suggested by the absence of secondary infection following the intraperitoneal injection into guinea pigs of the macerated viscera of 32 uninfected ticks after feeding (fig. 4) and in the one instance in

which the vaccine has been administered subcutaneously to man (9,500 M. I. D. of killed virus), slight local and no constitutional reaction followed.

3. COMPARISON OF TICK AND TISSUE VIRUS

Breinl (Breinl, 1924) in recent studies on the virus of typhus fever in lice, has emphasized the characteristics of louse virus as distinguished from animal-tissue virus. He observed in animals inoculated intraperitoneally with louse virus a shorter period of latency, a more irregular fever, and a higher death rate than among animals inoculated with tissue virus, and concluded that the irregular fever was due to the effect of large quantities of dead virus in the presence of live virus. Further, he concluded that the louse tissue contained far in excess of 100,000 doses of dead virus because of the inability to produce immunity with an amount of dead guinea-pig virus which contained this number of M. I. D. The observed differences between tissue virus and louse virus in typhus are seen to be somewhat analogous to those found by us between tick virus and guinea-pig virus in Rocky Mountain spotted fever. However, in interpreting our results we do not believe that the atypical infection following the use of tick virus is due to the combined action of dead and living organisms. We are rather inclined to the view that a decided change of the virus in quality or quantity or both has taken place. The afebrile and fatal infection following tick feeding *as well as injection of tick contents* (fig. 3) can not be readily accounted for on the ground of an interaction between live and dead virus, for it is highly improbable that large quantities of dead virus can be injected into an animal by means of tick feeding. We have recently observed in rabbits also a highly fatal infection following the feeding of infected ticks upon them. Yet, as everyone working with Rocky Mountain spotted fever knows, it is extremely rare to observe a fatal outcome in rabbits following the inoculation of the usual laboratory strain preserved by passage through guinea pigs.

In contrast with the mammalian host, the stages in the life cycle of the tick must influence the life of the contained virus, which strongly suggests the existence of a cycle in the life of the virus also. Phases of this cycle are herein indicated by the variations in virulence and infectivity (figs. 1, 2, and 3) of tick virus and the variation implied in the fact that killed-tick virus possesses strong immunizing power rarely exhibited by killed-tissue virus.

SUMMARY

1. In confirmation of earlier observations of previous workers, ticks of the species *D. andersoni* which have received the infection of Rocky Mountain spotted fever in the larval or nymphal stage retain it in the adult stage.

2. A 24-hour incubation at 37° C. of unfed hibernating nymphs and adults infected as larvæ and subsequent injection of emulsions of such ticks into guinea pigs give a higher percentage of positive infection than the injection of similar ticks not incubated.

3. Infection of Rocky Mountain spotted fever in adult ticks subjected to winter temperatures (32° F. or below) may be demonstrated by the production of immunity in guinea pigs following the injection of tick viscera immediately upon removal from cold temperatures, by a moderate but typical spotted fever following the injection of ticks after 24 hours incubation at 37° C., and by virulent spotted fever following tick feeding or the injection of ticks after feeding.

4. Control adult ticks free from all infection do not produce death or illness in guinea pigs by feeding nor by injection of such ticks after feeding.

5. One infected adult tick may contain after feeding, from 3,000 to 5,000 M. I. D. for a guinea pig. (See later studies, p. 51.)

6. An emulsion of infected fed adult ticks treated with 0.5 per cent phenol will protect guinea pigs against 1 c. c. of blood virus.

7. Nothing in the behavior of blood or tissue virus is comparable to the changes observed in tick virus.

NONFILTRABILITY OF TICK AND BLOOD VIRUS[11]

By R. R. Spencer, Surgeon, and R. R. Parker, Special Expert, United States Public Health Service

Attempts by Ricketts (Ricketts, 1907–c) to filter the virus of Rocky Mountain spotted fever as it occurs in the blood of infected animals resulted in failure of the virus to pass through. Similar tests by others (Wolbach, 1919–b), and by us have given uniformly negative results. So far as known to the writers, no one has heretofore undertaken the filtration of tick virus. Wolbach did not attempt its filtration because of unsatisfactory controls. He states:

> Preliminary transmission experiments made with thoroughly crushed tissues from proved infected ticks have deterred me from attempting filtration experiments, as uncertain results were obtained with the unfiltered crushed tissues in salt solution. The cause of failure to infect animals by tick tissues so treated has not been ascertained. In these experiments, using proved infective ticks, it was not possible to transmit the disease by injecting the thoroughly crushed tissues suspended in salt solution; and I have arrived at the tentative conclusion that the infectivity of the virus was destroyed by the procedure.

It was thought highly probable that tick virus which is readily demonstrated in infected fed adult ticks might prove filterable be-

[11] Reprint No. 982 from the Public Health Reports, vol. 39, No. 52, Dec. 26, 1924, pp. 3251–3255.

cause a salt solution emulsion of tick organs is less viscid and contains less albuminous material than does diluted guinea-pig serum, and also because, as we have shown, the virus present in adult ticks following feeding is highly concentrated. However, the intraperitoneal injection of the filtrates of the virus in citrated plasma and tick emulsions from Berkefeld "N" and "V" filters has failed to produce spotted fever in guinea pigs, although *Staphylococcus aureus*, an organism larger than *Dermacentroxenus rickettsi*, described as the causative agent of Rocky Mountain spotted fever, readily passed the "V" filters before and after the tests with Rocky Mountain spotted fever virus. In each instance the animals received larger volumes of the filtrate than animals inoculated with the unfiltered material.

FILTRATION NO. 1

January 2, 1924: Four infected adult ticks were removed from the ice box and, after incubating at 37° C. for two days, in order to induce feeding, were fastened in a wire gauze capsule to a guinea pig for three days.

January 7, 1924: The four partly fed ticks were removed and their viscera carefully crushed in 10 c. c. of salt solution. Guinea pigs Nos. 1 and 2 received intraperitoneally 1 c. c. each of the unfiltered tick organ emulsion. Guinea pigs Nos. 3 and 4 received intraperitoneally 2 c. c. each of the filtrate from a Berkefeld "N" candle which had been previously found to hold back a broth suspension of *Staphylococcus aureus*.

Results:

Guinea pig No. 1 developed typical spotted fever.
Guinea pig No. 2 developed typical spotted fever.
Guinea pig No. 3, no fever for 11 days.
Guinea pig No. 4, no fever for 11 days.
Guinea pigs Nos. 3 and 4, when subsequently tested for susceptibility by the injection of 1 c. c. of blood virus, developed typical spotted fever.

FILTRATION NO. 2

January 15, 1924: Six infected adult ticks were incubated and fed as in experiment No. 1. The internal organs were crushed in 14 c. c. of salt solution. Guinea pigs Nos. 5 and 6 received 1 c. c. each of the unfiltered emulsion. Guinea pigs Nos. 7, 8, and 9 received 5 c. c., 4 c. c., and 3 c. c., respectively, of the filtrate from the same candle used in experiment No. 1. The candle had been sterilized in the meantime.

Results:

Guinea pig No. 5 developed typical spotted fever.
Guinea pig No. 6 developed typical spotted fever.
Guinea pig No. 7, no fever for 13 days.
Guinea pig No. 8, no fever for 13 days.
Guinea pig No. 9, irregular fever; no external evidence of spotted fever.
Guinea pigs Nos. 7, 8, and 9, when subsequently tested for susceptibility by the injection of blood virus, developed typical spotted fever.

FILTRATION NO. 3

January 31, 1924: After incubation and three days' feeding on a guinea pig, the internal organs of 6 infected adult ticks were crushed in 15 c. c. of salt solution. Guinea pigs Nos. 10 and 11 received 1 c. c. each of the unfiltered emulsion, and Nos. 12 and 13 received 7 c. c. and 5 c. c., respectively, of the filtrate. The same Berkefeld candle was used as in previous experiments, and a 24-hour broth culture of *Staphylococcus aureus* was filtered immediately after the tick emulsion. One, two, and three cubic centimeters of the filtrate from the culture gave no growth in plain broth after 72 hours at 37° C.

Results:

Guinea pig No. 10 developed typical spotted fever.
Guinea pig No. 11 developed typical spotted fever.
Guinea pig No. 12, no fever for 11 days.
Guinea pig No. 13, no fever for 11 days.
Guinea pigs Nos. 12 and 13, when subsequently tested for susceptibility by the injection of blood virus, developed typical spotted fever.

FILTRATION NO. 4

February 19, 1924: In order to rule out a chemical or absorptive affinity for the virus on the part of the filter substance, an old candle was ground to a fine powder. One gram of the powder was suspended in 30 c. c. of distilled water and sterilized by boiling five minutes. After cooling, 2 c. c. of the sterilized suspension of the candle were added to 4 c. c. of guinea pig serum virus. This mixture was thoroughly shaken for 10 minutes and then centrifuged for one-half hour at about 2,000 revolutions per minute. On removal, a very clear layer of diluted serum lay above the powdered material packed at the bottom of the tube. Guinea pig No. 14 received intraperitoneally 1 c. c. from the top of the tube. Guinea pig No. 15 received

intraperitoneally 3 c. c. of the remaining clear solution. Guinea pig No. 16 received intraperitoneally the filter powder suspended in salt solution.

Results:

Guinea pig No. 14 developed typical spotted fever.
Guinea pig No. 15 developed typical spotted fever.
Guinea pig No. 16 developed typical spotted fever.

An experiment identical with the above was performed, with the exception that tick virus emulsion was substituted for serum virus. Similar results were obtained; all animals developed spotted fever.

FILTRATION NO. 5

September 24, 1924: A battery of six new Berkefeld "V" (coarse) filters was sterilized, and 15 c. c. of a 24-hour broth culture of *Staphylococcus aureus* was filtered through each. One, two, and three cubic centimeters of all the filtrates were planted into plain broth.

Filtrates from filters Nos. 1, 2, 5, and 6 gave growth in 24 hours. Those from Nos. 3 and 4 remained sterile five days. All filters were carefully marked for identification and resterilized the same day. Twenty-four cubic centimeters of the pooled citrated plasma from two spotted fever guinea pigs were diluted to 72 c. c., and about 10 c. c. was passed through each of the six filters.

Guinea pigs Nos. 17 and 18 received 2 c. c. of the diluted unfiltered serum. Guinea pigs Nos. 19, 20, 21, 22, 23, and 24 received 5 c. c. each of the filtrate from Nos. 1 to 6, respectively.

Results:

Guinea pig No. 17 developed typical spotted fever.
Guinea pig No. 18 developed typical spotted fever.
Guinea pig No. 19, no spotted fever for 12 days.
Guinea pig No. 20, no spotted fever for 12 days.
Guinea pig No. 21, no spotted fever for 12 days.
Guinea pig No. 22, no spotted fever for 12 days.
Guinea pig No. 23, no spotted fever for 12 days.
Guinea pig No. 24, no spotted fever for 12 days.
Pigs 19 to 24, receiving the filtrate, developed fatal spotted fever when later injected with blood virus.

FILTRATION NO. 6

September 26, 1924: The viscera of 10 infected engorged nymphs of *D. andersoni* were emulsified in 50 c. c. of salt solution. Pigs Nos. 25 and 26 received 1 c. c. each of the unfiltered emulsion. Pigs Nos. 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, and 37 and 38 received 2 and 4 c. c., respectively, of the filtrate from Berkefeld "V" filters Nos. 1 to 6.

Results:

Guinea pig No. 25: Developed typical spoptted fever.
Guinea pig No. 26: Developed typical spotted fever.
Guinea pig No. 27: No spotted fever for 12 days.
Guinea pig No. 28: No spotted fever for 12 days.
Guinea pig No. 29: No spotted fever for 12 days.
Guinea pig No. 30: No spotted fever for 12 days.
Guinea pig No. 31: No spotted fever for 12 days.
Guinea pig No. 32: No spotted fever for 12 days.
Guinea pig No. 33: No spotted fever for 12 days.
Guinea pig No. 34: No spotted fever for 12 days.
Guinea pig No. 35: No spotted fever for 12 days.
Guinea pig No. 36: No spotted fever for 12 days.
Guinea pig No. 37: No spotted fever for 12 days.
Guinea pig No. 38: No spotted fever for 12 days.
Guinea pigs Nos. 27 to 38 developed fatal spotted fever when inoculated with 1 c. c. of blood virus after 12 days.

FILTRATION NO. 7

September 29, 1924: Dilute citrated plasma pooled from two spotted fever guinea pigs was again filtered through the same six filters. Two guinea pigs received 1 c. c. each of the unfiltered solution and developed spotted fever. Six guinea pigs received 6 c. c. each of the filtrates from filters Nos. 1 to 6, respectively. None developed spotted fever for 10 days, but all developed it after the blood virus inoculation.

Immediately after this test, and before sterilization of the filters, a broth culture of *Staphylococcus aureus* was mixed with one-half its volume of normal guinea pig plasma, divided into six equal parts, and passed through the six filters. One cubic centimeter of the filtrates from each filter gave growth in plain broth.

SUMMARY

1. The virus of Rocky Mountain spotted fever as its occurs in the blood of guinea pigs and in emulsions of infected tick viscera (adults and nymphs) will not pass Berkefeld "N" and "V" filters.

2. Inoculation of filtrates of blood or tick virus does not produce immunity in guinea pigs.

3. The coarse "V" filters that hold back the virus of Rocky Mountain spotted fever will readily pass broth cultures of *Staphylococcus aureus.*

4. The failure of the virus to pass Berkefeld filters does not appear to be due to a chemical affinity for or adsorptive property of the material of which the filter candles are made.

VACCINATION OF MONKEYS AND MAN [12]

By R. R. SPENCER, Surgeon, and R. R. PARKER, Special Expert, United States Public Health Service

In a previous publication (see p. 20) we have shown that guinea pigs may be successfully vaccinated against Rocky Mountain spotted fever by injections of phenolized emulsions of tick virus. Data are now submitted which (1) prove that this vaccine will also protect monkeys and (2) suggest that it will confer immunity upon man.

PREPARATION OF THE VACCINE

As a rule, the production of a potent vaccine from tick emulsions is dependent upon a high concentration of virus in the ticks from which it is prepared. By the injection of graded dilutions of emulsions of infected tick viscera into guinea pigs, the minimal infectious dose of any given emulsion may be approximately determined. After many such titrations, employing fed and unfed infected ticks (*D. andersoni*) at all stages of the life cycle, it has been found that the highest concentrations of spotted-fever virus occur five to six days after the beginning of the adult feeding.[13] (See later observations, p. 52.)

Such ticks, usually in lots of 100, are permitted to feed three days on guinea pigs, then at once eviscerated one by one and ground in a mortar for 10 to 15 minutes with sterile sand and a few cubic centimeters of salt solution. By this procedure the internal organs are easily separated from the fragments of chitin, which quickly settle to the bottom and a fairly homogenous emulsion is obtained. The emulsion is next diluted with sufficient salt solution so that each cubic centimeter contains the equivalent of the viscera of two

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[12] From the Public Health Reports, vol. 40, No. 41, Oct. 9, 1925, pp. 2159–2167.

[13] *Rearing of infected ticks for the preparation of the vaccine.*—The rearing of adult ticks from which the vaccine is made is a prolonged and tedious process. Potent virus can not be obtained from the tick in the same stage in which it receives the infection. Virus ingested by larvæ does not increase appreciably in the engorged larvæ. It is not until the next stage, or nymphs, that any increase in amount or virulence is apparent, while in the succeeding adult stage the increase is often greater than in the nymphs. The ticks which serve as culture tubes must be infected as larvæ and then reared to adults; for it is in the adult ticks that the virus is most constantly of high virulence. It is, therefore, necessary to begin operations in the spring before that in which the vaccine is to be used. Adult males and females are selected from lots which have been reared in the laboratory and proved free from spotted fever and other infection by tests carried out during the earlier stages of the life cycle. The females are brought to full engorgement on rabbits, fertilization by the males occurring during the feeding. Each engorged female is placed in a separate pill box, assigned a lot number, and placed over moist sand. Egg deposition and hatching follow. The progeny of each of these females are carried forward as a unit. As larvæ and again as nymphs each lot is permitted to engorge on a host. An infected rabbit is used for feeding the larvæ and later a normal rabbit for the nymphs. After each engorgement a few ticks are injected intraperitoneally into guinea pigs to determine whether spotted fever infection has been acquired and to check again the absence of extraneous infection. After the nymphs have molted to adults they should be permitted to remain quiescent a few months, since experience has shown that recently molted adults do not as consistently yield as strong virus as those which are older.

or more ticks. At this stage the minimal infectious dose for guinea pigs is determined by the injection of graded dilutions, using two guinea pigs for testing each dilution. If either of the guinea pigs receiving one-thousandths of a tick fails to develop spotted fever, the material is not considered suitable for the preparation of a potent vaccine. (See later observations, p. 63.) Minimal infectious doses of one five-thousandths of a tick or more are preferable. The emulsion is now diluted with salt solution so that each cubic centimeter contains the equivalent of one tick, and phenol is added at the same time to make the final product contain 0.5 per cent of the preservative. The mixture is permitted to remain two or three days at room temperature. During this time a rather heavy precipitate forms and extraneous organisms are killed, as shown by subsequent anaerobic and aerobic sterility tests. The precipitate is best separated by slow centrifugation, since the emulsion does not pass readily through filter paper, and since its potency is destroyed if passed through a Berkefeld filter. The supernatant fluid, which is the material used as a vaccine, has a moderate turbidity (less than 200).

The precipitate after drying also has been shown to possess protective qualities if reemulsified and injected into guinea pigs. No further study of this fraction has been made up to the present time.

DOSAGE

We have found it difficult to determine the minimal protective dose for guinea pigs. A small amount of a given lot of vaccine may protect one guinea pig while an equal and sometimes larger amount of the same material fails to protect another animal of approximately equal weight. In comparing the potency of two batches of vaccine, one would expect higher protection from the lot which gave the lowest minimal infectious dose before the contained virus was killed with phenol. This is not necessarily the case. Such irregularities are not surprising, however, when we recall that so little is known of the various factors affecting the process and mechanism of immunity. Typical tests upon three batches of vaccine are given in Table 14.

TABLE 14.—*Typical tests on three batches of vaccine*

[Vaccine No. 130—Live virus titration = 1/1,000 c. c. (1/1,000 tick) M. I. D. Prepared April 2, 1925; tested April 15, 1925]

Pig No.	Dose of vaccine[1]	Result of blood virus injection (1 c. c.) 12 days later	Pig No.	Dose of vaccine[1]	Result of blood virus injection (1 c. c.) 12 days later
	C. c.			*C. c.*	
1	¼	Spotted fever. Recovered.	4	½	Spotted fever. Died.
2	¼	Spotted fever. Died.	5	1	Died from secondary infection.
3	½	Immune.	6	1	Immune.

[1] Each dose of vaccine given in cubic centimeters also represents the same fraction of a tick.

TABLE 14.—*Typical tests on three batches of vaccine*—Continued

[Vaccine No. 221—Titration=1/1,000 tick (M. I. D.). Prepared March 31, 1925; tested April 15, 1925]

Pig No.	Dose of vaccine	Result of blood virus injection (i. c. c.) 12 days later	Pig No.	Dose of vaccine	Result of blood virus injection (i. c. c.) 12 days later
	C. c.			*C. c.*	
7	¼	Immune.	10	½	Immune.
8	¼	Died early of secondary infection.	11	1	Do.
9	½	Immune.	12	1	Do.

[Vaccine No. 223—Titration=1/5,000 tick (M. I. D.). Prepared April 11, 1925; tested April 15, 1925]

13	¼	Spotted fever. Recovered.	16	½	Immune.
14	¼	Spotted fever. Died.	17	1	Do.
15	½	Immune.	18	1	Do.

Guinea pig No. 3 was protected by one-half cubic centimeter of vaccine No. 130, but the same amount did not protect guinea pig No. 4.

Guinea pig No. 7 was protected by one-fourth cubic centimeter of vaccine No. 221, but one-fourth cubic centimeter of vaccine No. 223 failed to protect guinea pigs Nos. 13 and 14, although this vaccine was prepared from an emulsion containing 5,000 M. I. D. per cubic centimeter as compared with 1,000 M. I. D. in vaccine No. 221.

In amounts smaller than one-fourth cubic centimeter the vaccine has frequently modified, but never completely prevented, the infection. On the other hand, 1 c. c. amounts have invariably protected guinea pigs when the minimal infectious dose of the live virus emulsion was one five-thousandth cubic centimeter. More concentrated lots of vaccine so prepared that the equivalent of two ticks was contained in each cubic centimeter have been used with good results. Higher concentration than this, however, yield emulsions of great turbidity.

The majority of our preparations have lost their protective quality after five or six weeks, but we have encountered one lot which fully protected guinea pigs in 1 c. c. amounts after six months' storage in the ice box. (See later observations, p. 70.)

Guinea pigs receiving two doses of 1 c. c. each have shown immunity to 1 c. c. of blood virus as long as eight months. Tests for longer periods were not made.

These tests merely indicate what may be expected as to the duration of immunity, the minimal protective dose, and the keeping qualities of the vaccine. At the present time our data are too meager to justify the making of generalizations.

VACCINATION OF MONKEYS

Tables 15 and 16 show complete protection of vaccinated monkeys against 1 c. c. of blood virus (500 to 1,000 M. I. D. per cubic centimeter for guinea pig) and against one-tenth cubic centimeter of tick virus (500 M. I. D.). All five control monkeys died showing a typ-

ical rash. A spleen emulsion of monkey No. 10 produced typical fever and symptoms when injected into guinea pigs. The eight vaccinated animals remained well.

TABLE 15.—*Vaccine No. 210—Vaccination of M. rhesus*

[Vaccine prepared February 19, 1925; injected subcutaneously. Titer=1/5,000 tick]

No.	First dose Feb. 25, 1925	Second dose Mar. 2, 1925	Immunity test Mar. 12, 1925	Result
1	Control		1 c. c. blood virus	Mar. 25, 1925.—Dead. Typical spotted fever and rash.
2	do	do	do	Do.
3	do	do	do	Mar. 22, 1925.—Dead. Typical spotted fever and rash.
4	1 c. c.	do	do	Apr. 4, 1925.—Active and healthy.
5	do	do	do	Do.
6	do	1 c. c.	do	Do.
7	do	2 c. c.	do	Do.
8	do	do	do	Do.

TABLE 16.—*Vaccine No. 219—Vaccination of capuchin monkeys*

[Vaccine prepared March 21, 1925; injected subcutaneously. Titer=1/1,000 tick]

No.	First dose Mar. 25, 1925	Second dose Apr. 1, 1925	Immunity test Apr. 11, 1925	Result
9	Control		1/10 c. c. tick virus	Apr. 21.—Dead. Typical spotted fever.
10	do	do	do	Do.
11	1.5 c. c. vaccine.	1.5 c. c. vaccine.	do	Remained well; discharge June 1.
12	do	do	do	Do.
13	do	do	do	Do.

EXPERIMENTAL VACCINATION OF MAN

In investigations of Rocky Mountain spotted fever or other disease transmitted by insect vectors, no attempt has been made, so far as we are aware, to protect man by inoculating material from an intermediate host. Breinl (Breinl, 1924) has shown that guinea pigs may be protected against typhus fever by injecting phenolized emulsions of infected lice, but the method was not applied to man.

Up to the present time a total of 34 people, chiefly laboratory and field workers in Rocky Mountain spotted fever and others whose occupations expose them to infection, have been vaccinated. Each lot of vaccine for human use was first tested for sterility, following the Hygienic Laboratory technique required for biologic products, and for protective quality and harmlessness by injection into guinea pigs.

The vaccine was administered subcutaneously in doses of 1 or 2 c. c. each at 5-day intervals. Some have received 2, some 3, and some 4 injections. No severe reactions have been encountered. The total number of M. I. D. for guinea pigs per vaccinated individual has varied from 2,000 to 17,500. The greatest number

used for one injection has been 10,000. The vaccines used have contained 1,000, 5,000, and 10,000 M. I. D. of killed virus per cubic centimeter.

Local redness, swelling, and heat, reaching a maximum within 48 hours, is the rule. Slight headache and muscular pains occurred in a few instances, but no elevations of body temperature developed, and all persons vaccinated carried on their duties as usual. The reactions following the first injection have been exceedingly mild, but in some individuals they increased in intensity and duration after the last injection, suggesting an increasing sensitiveness.

One person who gave a history of extreme sensitiveness to the bite of insects developed a general itching, with watering of the eyes, almost immediately following the first injection. For two weeks there appeared and disappeared several crops of an urticarial rash, accompanied by itching. The condition, however, did not interfere with the performance of his regular duties. An intradermal injection of one-tenth cubic centimeter of the vaccine given two weeks later was followed immediately by an urticarial wheal 1 inch in diameter.

DEMONSTRATION OF PROTECTIVE BODIES IN THE SERUM OF VACCINATED ANIMALS AND OF MAN

On May 22, 1924, 1 c. c. of serum from each of 12 normal Belgian rabbits was mixed with 1 c. c. of guinea pig blood virus and immediately injected intraperitoneally into guinea pigs. All these animals developed typical spotted fever, from which only one recovered, demonstrating that the normal rabbit sera contained no virus-neutralizing substances. The rabbits were subsequently each given subcutaneously three injections of vaccine (May 22, May 26, and June 13). On August 21, 2 rabbits having died of intercurrent infection, 1 c. c. of the serum of each of the remaining 10 was again mixed with 1 c. c. of blood virus and immediately injected into fresh pigs. The sera of two fresh rabbits were used as controls. Figure No. 7 gives the results of these inoculations. The sera of the controls (Nos. 19 and 20) and of one of the vaccinated rabbits (No. 21) did not apparently affect the virus. However, two of the guinea pigs (Nos. 25 and 28) developed no symptoms of fever and all the others gave a markedly delayed incubation period.

Figure No. 8 gives the result of injecting into guinea pigs mixtures of blood virus with (1) serum from vaccinated guinea pigs and (2) with serum from vaccinated man. This time the mixtures were permitted to remain one hour at room temperature before inoculating.

Guinea pigs Nos. 31, 32, and 33 were injected with mixtures of normal guinea pig serum and virus. Guinea pigs Nos. 34, 35, and

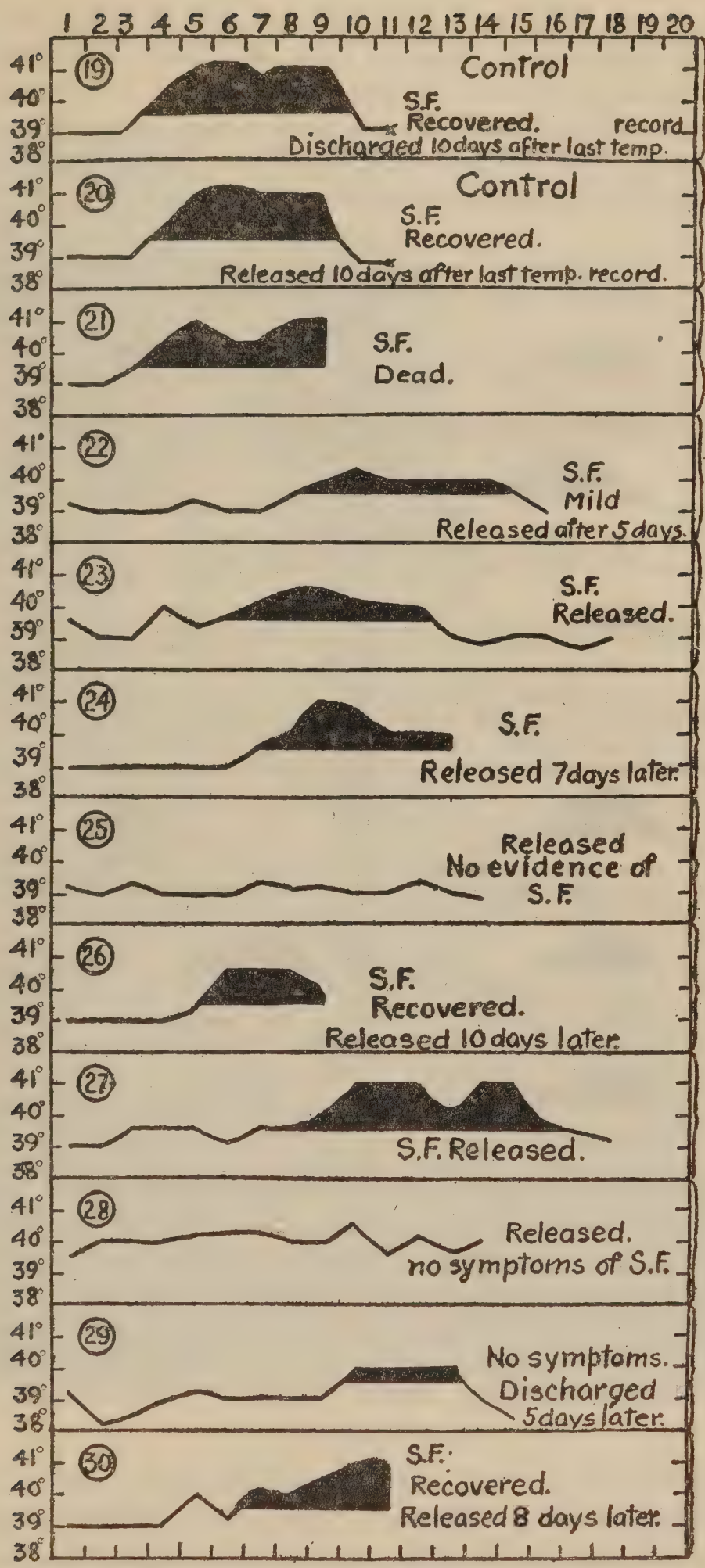

FIGURE 7.—Daily temperatures of guinea pigs injected with mixtures of serum from vaccinated rabbits and blood virus. (Temperatures above 39.6° C. are regarded as definite fever, and areas between this line and the temperature curve are shaded in black)

36 were injected with mixtures of normal human serum and virus. All six of these control animals died promptly of spotted fever. Guinea pigs Nos. 39 and 42 show that 0.5 c. c. of serum of a vaccinated guinea pig and of a vaccinated man neutralizes 0.2 c. c. of blood virus. The human serum had been tested before vaccination and showed no virus-neutralizing effect.

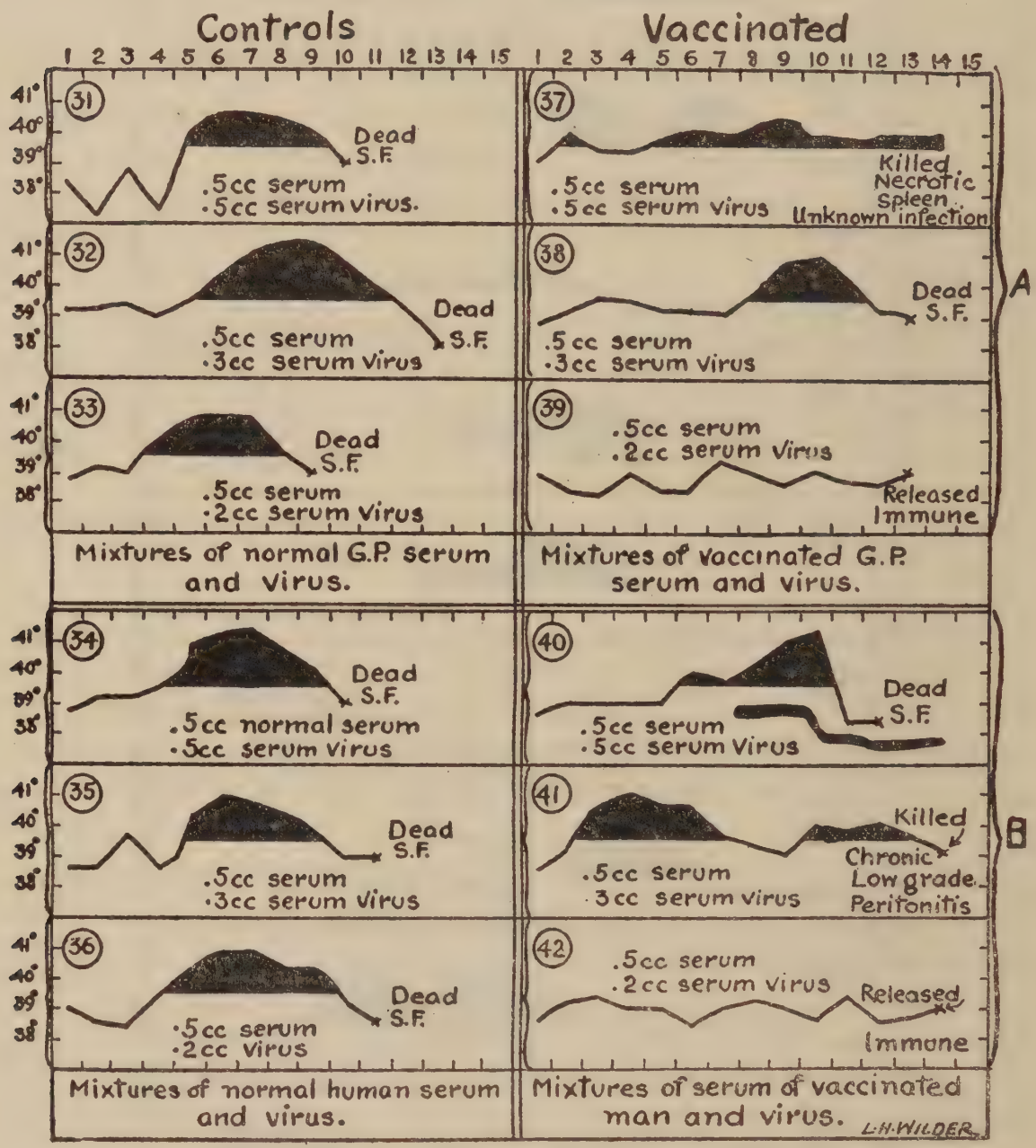

FIGURE 8.—Daily temperatures of guinea pigs injected with (A) mixtures of serum from vaccinated guinea pigs and decreasing amounts of blood virus, and (B) mixtures of serum from vaccinated man and decreasing amounts of blood virus

PROBABLE MODIFICATION OF ROCKY MOUNTAIN SPOTTED FEVER IN A VACCINATED PERSON

On April 8, 1925, E. O. E., of Stevensville, Mont., aged 43, engaged in cattle dipping for the Montana State Board of Entomology, was given 2 c. c. of vaccine No. 218. At the same time several other men were given injections from the same vial. Five days later E. O. E.

Plate 1.—Fatal case of Rocky Mountain spotted fever: Photograph taken about the eighth day. Many cases in the Bitter Root Valley, Mont., die before the rash has developed to this stage. (Courtesy of Surg. L. D. Fricks.)

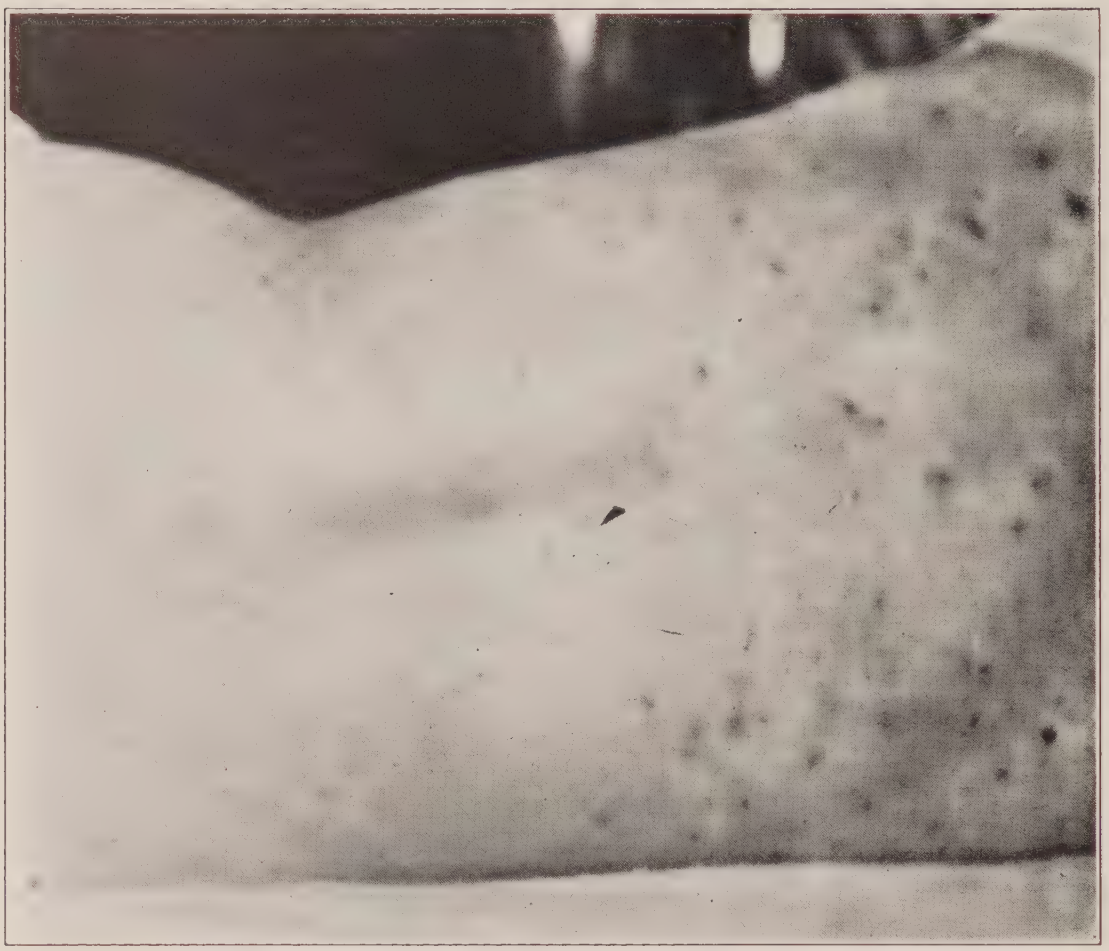

Plate 2.—Eighth day appearance of rash in a case of Rocky Mountain spotted fever, which was probably modified by vaccination

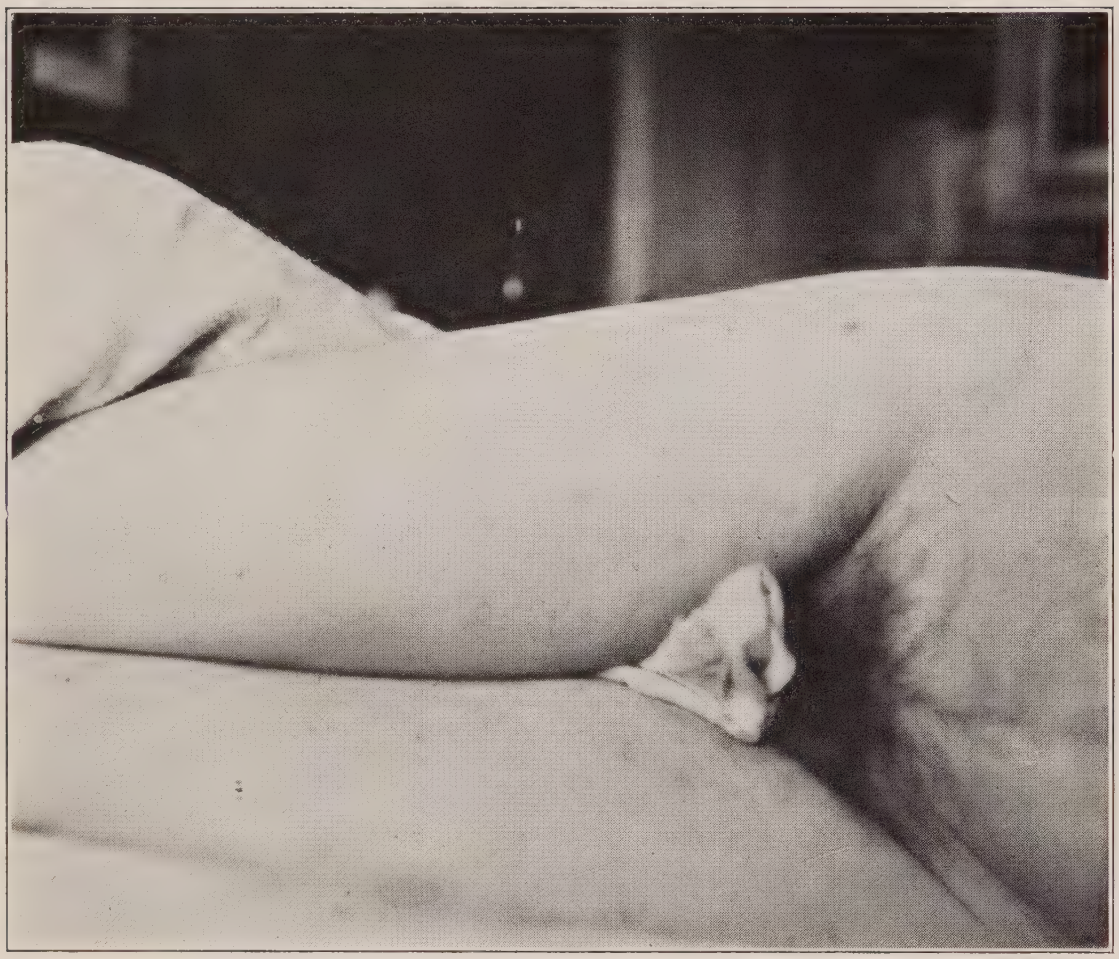

Plate 3.—Eighth day appearance of rash in a case of Rocky Mountain spotted fever, which was probably modified by vaccination

was given another injection of 1 c. c. in the opposite arm. On the morning of April 16, eight days after the first injection, he arose tired and drowsy, with a slight headache and muscular pains. The following day the symptoms were more severe, and in the evening, about 7 p. m., he had a chill and went to bed. On the 19th the family physician, Dr. W. P. Reynolds, of Stevensville, noticed small scattered hyperemic spots on the ankles and abdomen. On the 21st there was nosebleed and the temperature had reached 103.8° F. A diagnosis of Rocky Mountain spotted fever was made, although no definite history of tick bite could be obtained. The patient's occupation, however, had continually exposed him to the bites of partially fed adult ticks which he had been removing from cattle and horses by "hand picking." Such fed ticks can infect far more readily than unfed ticks. This has been previously shown by us experimentally. (See p. 6.)

The interesting features in this case are:

1. The course of the disease was mild, and the patient was never considered in danger.

2. Although convalescence was prolonged, the patient recovered. Four other cases—all that occurred this year in the Bitter Root Valley—were all fatal within 10 days.

3. The rash was scattered and far less marked than in any other cases we have seen in this region. (See pls. 2 and 3.)

4. Three guinea pigs injected with blood from the patient failed to show spotted fever. This has happened, however, in other typical cases, but never in those of a severe character.

It is, of course, impossible to state that the vaccine modified the course and severity of the infection. Vaccine No. 218 was prepared on March 18, 1925, from a tick emulsion which gave a titer of one one-thousandth tick as the M. I. D. On March 25 it protected guinea pigs in one-half cubic centimeter amounts.

The time required for an immunity to develop has varied considerably in vaccinated guinea pigs. We have regarded 10 days as a minimum period. We are therefore unable to draw any definite conclusions as to the efficiency of the vaccine from this case, but believe the circumstances as reported are worthy of note.

We see no reason why injections may not be repeated each spring in persons whose occupations definitely expose them to infection. It would be impracticable to vaccinate the general population of the spotted-fever region, but it is believed that the vaccine affords a means of protection for all those necessarily exposed to the infection and who choose to avail themselves of it. This would include residents and vacationists in badly infected areas, laboratory and field workers in Rocky Mountain spotted fever, foresters, lumbermen, sheep herders, surveyors, hunters, prospectors, fishermen, and others who engage in out-of-door pursuits.

SUMMARY

1. The technique for the preparation of a protective vaccine against Rocky Mountain spotted fever from infected adult ticks is given.

2. The potency, keeping qualities, and duration of immunity induced by this vaccine have been estimated within certain limits.

3. The vaccine will protect guinea pigs, rabbits, and monkeys.

4. The vaccine has been administered to 34 adults with no severe reactions.

5. Virus-neutralizing substances can be demonstrated in the serum of vaccinated guinea pigs, rabbits, and man.

6. The course and outcome of a case of Rocky Mountain spotted fever developing eight days after the first dose of vaccine suggests that the infection was modified.

A STUDY OF THE RELATIONSHIP BETWEEN THE PRESENCE OF RICKETTSIALIKE ORGANISMS IN TICK SMEARS AND THE INFECTIVENESS OF THE SAME TICKS[14]

By R. R. PARKER, Special Expert, and R. R. SPENCER, Surgeon, United States Public Health Service

Observations upon Rocky Mountain spotted fever infection in the tick vector (*Dermacentor andersoni* Stiles) have shown repeatedly that if of two groups of hibernating adult ticks from the same infected lot, the ticks of one group were examined without feeding and those of the other after feeding, those of the fed group would show (*a*) a greater percentage of ticks in which rickettsiæ can be demonstrated, (*b*) a tremendous increase in the number of rickettsiæ in the individual ticks, and (*c*) a much higher percentage of infective ticks. In fact, in *unfed* infected adults the rickettsiæ associated with Rocky Mountain spotted fever are often very difficult, or impossible, to find by smear preparations, especially if kept at cold temperature, whereas in *fed* ticks of the identical lot they are usually very abundant.

Our observations tabulated below were made upon individual adult ticks, part of them wild and of unknown history, and part reared, infected stock lots, the histories of which were known for at least one full laboratory generation. The latter were infected as larvæ and tested as the resultant adults of the same generation.

Because of our evidence that both infectiousness of spotted fever virus and the presence of rickettsiæ can be more accurately determined in ticks that have ingested blood, all adults used (except the controls under "A" below) were permitted to feed for two or sometimes three days prior to dissection.

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[14] Reprint No. 1067 from the Public Health Reports, vol. 41, No. 11, Mar. 12, 1926, pp. 461–469.

For the demonstration of the rickettsiæ we depended upon the examination of smears of pieces of tick tissue from the salivary glands, brain, intestines, reproductive organs, Malpighian tubules, and sucking muscles (muscles of the cheliceræ). These smears were fixed for one-half hour in Regaud's solution,[15] and stained in Giemsa's solution. The remaining viscera of each tick were inoculated intraperitoneally into a guinea pig, thus affording an opportunity to compare smear results with the infectiousness of the same ticks.

A. ADULT TICKS REARED AND INFECTED IN THE LABORATORY (1923 SERIES)

Table 17 presents the results of smear examinations and viscera inoculations with both fed and unfed adult ticks of the known infected lot, 797 B.[16] All ticks in Table 17, except 12 controls (Nos. 1 to 6 and 62 to 67) were first fed on an animal host in order to "reactivate" the virus, next examined for rickettsiæ by means of smear preparations, and finally tested for infectiveness by inoculating the remaining viscera into a guinea pig. For the smear preparation, parts of the salivary glands, brain, intestines, reproductive organs, and Malpighian tubules were used.

TABLE 17.—*Presence of rickettsialike organisms in laboratory-reared, infected adult ticks (lot 797 B) compared with the results of injecting guinea pigs with emulsions of the same ticks (section A of text)*

UNFED ADULT TICKS

Tick No.	Date tested	Stained smears					Result of guinea-pig inoculation
		Brain	Salivary gland	Intestines	Reproductive organs	Malpighian tubule	
1	July 31, 1923	−	−	−	−	−	Negative.
2	-----do-------	−	−	+	−	−	Do.
3	-----do-------	−	−	−	−	−	Do.
4	-----do-------	+	−	+	+	+	Died in 6 days. Cause undetermined.
5	-----do-------	−	−	−	−	−	Negative. Subsequently immune.
6	-----do-------	−	−	−	−	−	Do.
	FED ON CALF FROM JULY 21 TO AUGUST 2						
7	Aug. 6, 1923	−	−	−	−	−	Spotted fever.
8	-----do-------	+	+	+	−	+	Do.
9	-----do-------	−	−	+	−	+	Do.
10	-----do-------	−	−	−	−	−	Do.
11	-----do-------	+	+	+	−	+	Negative.
12	-----do-------	−	−	−	−	−	Do.
13	Aug. 7, 1923	+	+	+	−	+	Spotted fever.
14	-----do-------	+	+	+	−	+	Do.
15	-----do-------	−	−	−	−	−	Negative.

[15] Potassium bichromate (3 per cent) ---------- 100 parts
Formalin (40 per cent) ---------- 25 parts

[16] History of lot 797 B:
May 26, 1922.—Engorged female collected from a horse.
July 1, 1922.—Larvæ began hatching from eggs deposited by female.
Sept. 12, 1922.—Larvæ began feeding on an infected Belgian rabbit; inoculated 5 days previously with a laboratory strain of spotted fever.
Oct. 1, 1922.—Engorged larvæ began molting to flat nymphs.
Apr. 14, 1923.—Normal Belgian rabbit infested with flat nymphs.
May 6, 1923.—Engorged nymphs tested by and found infected inoculation in a guinea pig.
June 2, 1923.—Engorged nymphs began molting to flat adults.

TABLE 17.—*Presence of rickettsialike organisms in laboratory-reared, infected adult ticks, etc.*—Continued

Tick No.	Date tested	Stained smears					Result of guinea-pig inoculation
		Brain	Salivary gland	Intestines	Reproductive organs	Malpighian tubule	
		FED ON JACK RABBIT FROM JULY 21 TO AUGUST 1					
16	Aug. 7, 1923	+	+	+	−	+	Spotted fever.
17	do	−	−	−	−	−	Do.
18	do	−	−	+	−	−	Do.
19	do	+	+	+	−	+	Do.
20	do	+	+	+	−	−	Do.
21	Aug. 11, 1923	+	+	+	−	−	Do.
22	do	−	−	−	−	−	Do.
23	do	+	+	+	+	+	Negative.
24	do	−	−	−	−	−	Spotted fever.
25	do	+	+	+	+	+	Do.
		FED ON HORSE FROM JULY 21 TO AUGUST 8					
26	Aug. 8, 1923	+	+	+	+	+	Spotted fever.
27	do	+	+	+	−	+	Do.
28	do	+	+	+	−	+	Do.
29	do	−	−	−	−	−	Negative.
30	do	+	+	+	−	+	Spotted fever.
31	do	+	+	+	−	+	Do.
32	do	+	+	+	+	+	Do.
33	do	−	−	−	−	−	Negative.
34	do	−	−	−	−	−	Do.
35	do	+	+	+	+	+	Spotted fever.
36	do	−	−	−	−	−	Do.
		FED ON BELGIAN RABBIT FROM JULY 21 TO AUGUST 7					
37	Aug. 9, 1923	+	+	+	−	+	Spotted fever.
38	do	+	+	+	−	+	Do.
39	do	+	+	+	−	+	Do.
40	do	+	+	+	−	+	Do.
41	do	+	+	+	−	+	Do.
		FED ON SNOWSHOE RABBIT FROM JULY 23 TO AUGUST 9					
42	Aug. 11, 1923	−	−	−	−	−	Spotted fever.
43	do	+	+	+	−	−	Do.
44	do	+	+	+	+	+	Do.
45	do	+	+	+	−	+	Do.
46	do	−	−	−	−	−	Do.
47	do	+	+	−	+	+	Do.
		FED ON SHEEP FROM JULY 25 TO AUGUST 9					
48	Aug. 13, 1923	+	+	−	−	+	Spotted fever.
49	do	+	+	+	+	+	Do.
50	do	+	+	+	−	+	Negative.
51	do	+	+	+	+	+	Spotted fever.
52	do	+	+	+	+	+	Do.
53	do	+	+	+	+	+	Do.
54	do	−	−	−	−	−	Died in 2 days. Valueless.
55	do	−	−	−	−	−	Negative.
		FED ON GUINEA PIG FROM AUGUST 1 TO AUGUST 20					
56	Aug. 20, 1923	+	+	+	+	+	Negative.
57	do	+	+	+	+	+	Spotted fever.
58	do	+	+	+	+	+	Do.
59	do	+	+	+	+	+	Do.
60	do	+	+	+	+	+	Negative.
61	do	+	+	+	+	+	Spotted fever.
		UNFED ADULT TICKS					
62	Aug. 29, 1923	−	−	−	−	−	Negative.
63	do	−	−	−	−	−	Do.
64	do	−	−	−	−	−	Do.
65	do	−	−	−	−	−	Do.
66	do	−	−	+	−	+	Do.
67	do	+	+	+	−	+	Do.

Initial tests of unfed control ticks.—On July 21, six unfed ticks (Nos. 1 to 6) were dissected, smeared, and inoculated. Rickettsiæ were found in only two of them and none of the inoculated guinea pigs developed spotted fever. The rickettsiæ occurring in such nonfever-producing ticks (Nos. 2 and 4) were always morphologically indistinguishable (coccoidal, short bacillary and diplo-bacillary forms) from those found in the fed ticks which did produce spotted fever.

Tests of fed ticks.—Fifty-five ticks (Nos. 7 to 61) were fed on various hosts as indicated in the table. The following tabulation shows the relationship found between the presence or absence of rickettsiæ in the smears and the infectiveness of the viscera of these 55 fed ticks:

Rickettsiæ in smears		Results of inoculation of remaining viscera of indentical ticks	
Present	Absent	Spotted fever	Negative
40	----------	35	[1] 5
----------	15	[2] 8	7

[1] Nos. 11, 23, 50, 56, 60.
[2] Nos. 7, 10, 17, 22, 24, 36, 42, 46.

It is evident that of 40 ticks in which rickettsiæ were present, 35 produced spotted fever and 5 did not, and that of 15 in which rickettsiæ were not demonstrated 8 produced spotted fever and 7 did not. Comparing the initial control tests upon the unfed ticks with the fed ticks, marked increases are observed in the proportion of ticks showing rickettsiæ and the proportion of ticks producing spotted fever following inoculation. The percentage of ticks with rickettsiæ was increased from 33.33 to 72.72, and that of infective ticks (immunity-producing ticks excluded) from zero to 78.18. We observed also the usual tremendous increase in the number of rickettsiæ in individual tick smears of the fed group as compared with the unfed.

Final tests of unfed control ticks.—Control tests upon the unfed ticks were again made on August 29, following the termination of the experimental feedings. This was done in order to rule out the possibility that the increase in the number of rickettsiæ noted in smears, and the increase in the infectiveness of the viscera of fed ticks (Nos. 7 to 61) might have been due to some environmental condition other than the tick feeding or some other unrecognized influence to which the rickettsiæ in both fed and unfed adults were exposed subsequent to the initial tests, and prior to the tests upon the fed ticks. Of these six unfed ticks (Nos. 62 to 67) none produced spotted fever upon inoculation, and only two showed rickettsiæ in the smears,

these results being identical with those of the initial control test. Therefore, the increase in rickettsiæ as well as the infectiousness in ticks Nos. 7 to 61 was manifestly brought about by the ingestion of blood and attendant conditions. The rickettsiæ in these latter unfed controls were, like those in controls Nos. 2 and 4, morphologically indistinguishable from those found in the fed ticks.

B. WILD ADULT TICKS (1923 SERIES)

It is interesting to compare the results secured with wild ticks with those just given for the known infected lot, 797B. The unfed wild ticks were collected both from the east and west sides of the Bitterroot Valley, the latter being an area of severe infection, whereas no human cases have ever been shown to have originated on the east side, nor have we ever recovered a frank infection from east-side ticks. As before, all ticks were fed on guinea pigs for two days prior to dissection.

Although smears and inoculations were made from 800 ticks we have tabulated in Table 18 only a small selected group of these east and west side wild adult ticks which show definite rickettsiæ. Many of these showed rickettsiæ similar to those of the infected group in the smears of one or more tissues, but were not infective upon inoculation.

TABLE 18.—*Presence of rickettsialike organisms in miscellaneous adult ticks from nature, compared with result of injecting guinea pigs with emulsions of the same ticks (section B of text)*

Tick No.	Date tested	Stained smears					Result of guinea-pig inoculation
		Brain	Salivary gland	Intestines	Reproductive organs	Malpighian tubule	
FED ON GUINEA PIG JUNE 26 AND 27 (EAST SIDE)							
1	July 17, 1923	−	−	−	+	−	Negative.
2	-----do-----	−	−	−	+	+	Do.
3	-----do-----	−	−	−	+	−	Do.
4	-----do-----	−	−	−	+	+	Do.
5	July 19, 1923	−	−	−	+	−	Do.
6	-----do-----	−	−	−	+	−	Do.
7	-----do-----	−	−	−	+	−	Do.
FED ON GUINEA PIG JULY 7 TO 9 (WEST SIDE)							
8	July 26, 1923	−	−	−	+	−	Negative.
9	-----do-----	−	+	−	+	+	Do.
10	-----do-----	+	+	+	+	+	Do.
11	-----do-----	−	−	−	+	+	Do.
FED ON GUINEA PIG JULY 13 TO 15 (WEST SIDE)							
12	July 26, 1923	−	−	−	−	+	Negative.
13	-----do-----	−	−	−	+	−	Do.
14	-----do-----	−	−	+	+	+	Do.
15	July 31, 1923	+	+	+	+	+	Do.
16	-----do-----	−	−	−	+	+	Do.
17	-----do-----	−	−	−	+	+	Do.
18	July 23, 1923	+	+	+	+	+	Do.
19	-----do-----	+	+	+	+	+	Do.
20	-----do-----	−	−	+	−	+	Do.
21	-----do-----	+	+	+	+	+	Do.
22	July 24, 1923	−	−	−	+	+	Do.
23	-----do-----	+	−	+	+	+	Do.
24	-----do-----	−	−	−	+	+	Do.
25	-----do-----	−	−	−	+	+	Do.
26	July 25, 1923	+	+	+	+	+	Do.
27	-----do-----	+	+	+	+	+	Do.
28	-----do-----	+	+	+	+	+	Do.

C. ADULT TICK TESTS (1925 SERIES)

Two years after the above tests had been performed, two more series of 100 ticks each, infected and uninfected, were similarly tested, with the exception that smears of the sucking muscles (muscles of the chelicerae) were made in addition to the smears of the other tick tissues. This was done because rickettsiæ in large numbers are so frequently present in the muscles of infected adults both before and after feeding, especially under the latter condition.

The 100 ticks of the infected series were from several lots reared in the laboratory. Their histories were analogous to the history of lot 797 B, having been infected as larvæ during the summer of 1924, reared to adults by fall, and having passed the following winter as unfed adults. The 100 ticks of the noninfected series were collected from the east side of the Bitterroot Valley during the spring of 1925. All ticks of both series were fed on guinea pigs for three days in groups of about 25 to an animal, then dissected, the smears of the six tissues made, and, finally, the remaining viscera of each tick injected into a guinea pig. Healthy male animals weighing 500 grams or over were used exclusively.

Table 19 gives the occurrence and distribution of rickettsiæ in the two series.

TABLE 19.—*Occurrence and distribution of rickettsiæ in wild and in reared infected adult ticks of D. andersoni (section C of text)*

[1925 Series]

RICKETTSIÆ OCCURRENCE

	Present in—	Absent in—
100 adult ticks from east side of Bitterroot Valley [1]	42 ticks	58 ticks.
100 reared infected adults [2]	60 ticks	40 ticks.

RICKETTSIÆ DISTRIBUTION

	42 noninfected ticks	60 infected ticks
Rickettsiæ in muscle smears	5 ticks	54 ticks.
Rickettsiæ in brain smears	do	55 ticks.
Rickettsiæ in salivary-gland smears	3 ticks	47 ticks.
Rickettsiæ in intestine smears	4 ticks	52 ticks.
Rickettsiæ in reproductive organs smears	36 ticks	48 ticks.
Rickettsiæ in Malpighian tubule smears	4 ticks	52 ticks.

[1] None of the 100 guinea pigs injected with viscera of these ticks developed spotted fever.
[2] 65 guinea pigs injected with tick viscera of this lot gave evidence of spotted fever.

In the noninfected east-side group smears of 42 ticks (42 per cent) showed rickettsiæ. Thirty-six of these showed these organisms in the reproductive organs, while in only eight ticks were they present in any of the other tissues. In the infected group, on the other hand, rickettsiæ were present in 60 ticks (60 per cent); and instead of be-

ing largely restricted to the reproductive organs they were usually distributed in large numbers throughout the tissues.

In the noninfected group the rickettsiæ stained, as a rule, purple or pink and were generally filiform organisms. However, in many instances they closely resembled, and to us were indistinguishable from the deep-blue staining, short bacillary and diplo-bacillary forms found in the infected group.

In the muscle tissue of the infected group the rickettsiæ were very numerous, stained blue, and frequently were arranged in rows packed *between* the muscle fibers (not intracellular).

Of the 100 ticks from the east side of the Bitterroot Valley not one produced spotted fever when the viscera were injected into guinea pigs, nor were any of the animals subsequently immune to 1 c. c. of guinea pig's blood virus.

Of the infected group which is further analyzed in Table 20, 60 produced spotted fever and 5 (a total of 65 per cent) gave evidence of infection by immunizing the injected guinea pigs against a subsequent injection of blood virus. In some individual lots of this infected group more than 90 per cent gave evidence of infection, in others only 33⅓ per cent.

TABLE 20.—*Comparison of results of guinea pig inoculation of the viscera of 100 reared, infected adult ticks with the presence of rickettsiæ in the smears of same*

[1925 series]

100 REARED INFECTED ADULT TICKS

60 ticks with rickettsiæ in one or more organs				40 ticks in which rickettsiæ could not be found			
Result of guinea-pig injection				Result of guinea-pig injection			
Evidence of infection		No evidence of infection		Evidence of infection		No evidence of infection	
Spotted fever	Immunity	Negative	Death from intercurrent infection	Spotted fever	Immunity	Negative	Death from intercurrent infection
54	0	5	1	6	5	27	2

Sixty ticks showed rickettsiæ in one or more organs. Five of these did not produce spotted fever although the organisms appeared to be identical with those in ticks that did produce the disease.

Among the 40 ticks in which rickettsiæ were not found, 6 gave spotted fever and 5 immunized the animals injected.

It is evident, then, in testing this group of adult ticks, all infected when larvæ with spotted fever virus, that rickettsiæ could not be demonstrated in the smears of 11 of 65 ticks (16.92 per cent) defi-

nitely shown to have contained spotted-fever virus by the injection of the viscera of the identical ticks into guinea pigs, and further that rickettsiæ indistinguishable from those associated with spotted fever were found in the smears of 5 of 32 ticks (15.62 per cent) that did not produce any evidence of spotted fever when similarly inoculated.

SUMMARY AND DISCUSSION

The data as presented show the following: (1) That, although of known infected adult ticks the majority of those containing rickettsiæ were infective, yet of each lot tested a small group of noninfective ticks contained rickettsiæ morphologically identical, while still another small group was infectious though the tick smears were entirely free of organisms. (2) That of wild ticks from a known infected area a considerable proportion contained rickettsiæ indistinguishable from those associated with spotted fever, and that the smear and inoculation results of such ticks were parallel with those of the known infected group. (3) That a small proportion of wild ticks from a supposedly uninfected area contained similar rickettsiæ, but none caused infection.

It is difficult to account for the noninfective rickettsiæ, which were present in part of the known infected, laboratory-reared ticks (Tables 17 and 20) and which exhibited a morphology identical with that of the rickettsiæ in fever-producing ticks of the same group. They may represent an avirulent phase of the spotted fever virus, although the nonpathogenic nature of these bodies can not, of course, be ruled out. This accords with previous observations (see p. 5) of tick virus in a similar lot of known infected ticks by which we demonstrated various degrees of virulence for guinea pigs ranging from a noninfective or an immunizing phase in *unfed*, æstivating or hibernating ticks to an active highly virulent phase *following feeding*. The term "reactivation" has been used to designate this transition (see p. 7) which has been repeatedly observed in known infected lots. For example, in recently infected larvæ, the virus is present but is noninfective unless massive doses are used (5 engorged larvæ very rarely infect; 25 usually, but not always cause infection, often of a mild character); in the resultant *unfed* hibernating nymphs the virus is present either in a noninfective or immunizing phase, but in the *fed* nymphs it has acquired marked virulence; a noninfectious or immunizing phase is again encountered in the resultant *unfed*, æstivating, or hibernating adults, but in the *fed* adults a high degree of virulence has been reacquired.

In presenting these observations we realize that the relatively small part of the tissue of a tick represented by our smear prepara-

tions can not be taken as absolute evidence of the absence of rickettsiæ from the entire tick. However, it is at least reasonable to believe that they were few in number, since the test ticks had all ingested blood and the rickettsiæ had thus been afforded, as we have shown, the most favorable conditions for multiplication and distribution throughout the various tissues. There is, of course, the possibility that they were present in an unrecognized form.

CERTAIN CHARACTERISTICS OF BLOOD VIRUS[17]

By R. R. Spencer, Surgeon, and R. R. Parker, Special Expert, United States Public Health Service

The experiments outlined below suggest that some of the virus in the blood of animals infected with Rocky Mountain spotted fever is inseparable from the red and white blood cells, that it has a strong affinity for the same cells of normal animals, that it can be easily separated from the platelets, and that it exists in a form not readily demonstrated by our present methods of staining. In this connection it is interesting to note that Kusama (quoted by Segal) and Segal (Segal, 1922), reported that the virus of typhus fever was not associated with leucocytes or erythrocytes, but with the platelets.

The apparent disparity between the number of demonstrable organisms in the blood of animals infected with Rocky Mountain spotted fever, and the high degree of infectiousness of the same blood has often been noted.

Ricketts (Ricketts 1909a), for example, saw in the blood serum of infected man, monkeys, and guinea pigs an occasional "diplococcus-like body with an eosin-staining intermediate substance." These bodies could be "found in any preparation of infected blood during a search of not more than a half hour's duration." Ricketts has also shown that 0.7 c. c. of red cells after the tenth washing was infectious, while 10 c. c. of the supernatant fluid after the tenth washing was not infectious. White cells after the third washing were infectious in 5 c. c. amounts. Smaller amounts of cell suspensions were not injected and the amount of the dilution after each washing was not given.

Wolbach (Wolbach 1919b) states that "the demonstration of the parasite in the circulating blood is extremely difficult."

Connor (Connor 1924c) by diluting spotted-fever serum five times with salt solution and centrifuging (2,000 revolutions per minute) for six hours was able to demonstrate in smears of the residue a few organisms identical with forms in tick and animal tissue described by Wolbach and Nicholson (Nicholson 1923). Connor says: "The unusual virulence of the blood (0.001 c. c. often being infectious)

[17] Reprint No. 1105 from the Public Health Reports, vol. 41, No. 35, Aug. 27, 1926, pp. 1817–1822.

seems somewhat incompatible with the exceedingly small number of organisms found."

Experiments

Experiment No. 1: Effect of high-speed centrifugation on virus in cell-free serum.—The blood serum of two spotted-fever-infected guinea pigs was pooled and a sample removed to determine the minimal infectious dose by injecting graded dilutions into guinea pigs (two animals to each dilution).

The remaining pooled serum was then centrifuged at 8,800 revolutions per minute for 15 minutes in a Leune centrifuge. One cubic centimeter of the top was carefully removed and used for graded injections into animals as before. All the remaining fluid, except 1 c. c. at the bottom, which contained a small amount of sediment, was discarded. This residue was similarly injected into animals in graded dilutions.

Result.—The serum before centrifugation produced spotted fever in both animals receiving 1/500 c. c. but not in those receiving less amounts.

The top serum of the centrifuged portion produced spotted fever in guinea pigs receiving 1/10 c. c. but not in less amounts.

Injection of the lower centrifuged portion produced spotted fever in one of the guinea pigs receiving 1/100 c. c. but not in the other nor in those receiving less amounts.

Centrifugation for a longer period than 15 minutes was inadvisable because of the great amount of heat produced by the high-speed machine. The heat was probably responsible for the decrease in infectivity of the centrifuged sediment.

In smears made from the top serum and stained with Giemsa's solution no organisms could be found. A few scattered rickettsia-like forms could be found in smears of the lower portion.

Experiment No. 2: Association of the virus with washed red cells.—The red cells of 12 c. c. of blood of a spotted-fever-infected guinea pig were washed three times with salt solution. The lower half only of the packed cells was then drawn off with a fine capillary pipette, care being taken not to draw up the thin top coat containing leucocytes and platelets. The pure red cells were then diluted to the original blood concentration, and 1 c. c. was used for graded dilutions and injected into guinea pigs. The remaining red cells were washed by centrifugation nine times more. Only three minutes were required for complete throwing down of the red cells at a speed of 1,200 revolutions per minute, and the clear supernatant salt solution was drawn off each time so that the packed red cells were left in a volume of less than 5 c. c. Salt solution sufficient to make a total volume of 40 c. c.

was added after each washing, and the cells were thoroughly mixed. This method of washing was followed in all subsequent tests.

Results.—After the third washing the injection of 1/10 c. c. of red cells (diluted to the original volume) produced typical spotted fever in each of two guinea pigs and a moderate fever developed in one of two animals receiving 1/100 c. c. of cells. Smaller amounts failed to give fever.

Two guinea pigs receiving 4 c. c. each of the supernatant salt solution of the twelfth washing were negative for 12 days and subsequently were found susceptible by reinoculation of blood virus.

Guinea pigs receiving 1 c. c. each of red cells (diluted to the original volume of the blood) after the twelfth washing and one of the pigs receiving 1/10 c. c. developed typical spotted fever. Less amounts failed to give fever. We have found it impossible to demonstrate organisms of any kind within the red cells either in fresh preparations or in smear preparations stained with Giemsa's solution.

Experiment No. 3: Association of the virus with washed white blood cells.—An exudate of white cells obtained by injecting aleuronat into the peritoneal cavity of an infected guinea pig was suspended in 10 c. c. of salt solution and washed twelve times in the same manner as in the case of the red blood cells. The supernatant fluid after each centrifugation could be poured off, leaving a volume of less than 1 c. c. of white cells. The washing of these cells was therefore more thorough than that of the red cells.

Result.—Guinea pigs receiving 1/100 c. c. of unwashed cells developed spotted fever. Less amounts failed.

Guinea pigs receiving 1 c. c. and one animal receiving 1/10 c. c. of the white cells, after the twelfth washing, developed spotted fever. Less amounts were unsuccessful.

Experiment No. 4: Adsorption of virus with normal red cells.—Five cubic centimeters of a cell-free serum obtained from an infected guinea pig was mixed with an equal volume of a suspension of washed normal red blood cells. After thorough agitation the mixture was permitted to stand one-half hour at room temperature. The red cells were then washed ten times as in previous tests and injected as before in graded amounts.

Result.—The two guinea pigs receiving 1 c. c. and the two receiving 1/10 c. c. of the washed cells developed spotted fever.

Experiment No. 5: Adsorption of virus with normal white blood cells.—Five cubic centimeters of cell-free guinea-pig serum virus was mixed with equal amounts of a suspension of normal guinea-pig leucocytes (one-half gram of moist leucocytes in 5 c. c. of salt solution). The mixture was thoroughly shaken and permitted to stand one-half hour at room temperature and then washed ten times by

centrifugation. The washed cells were then diluted to the original volume of 5 c. c. and injected into guinea pigs.

Result.—Both animals receiving 1/10 c. c. as well as the two receiving 1 c. c. of the washed cells developed spotted fever.

Experiment No. 6: Association of virus with platelets.—(*a*) A 5 c. c. suspension of platelets obtained from 10 c. c. of blood of a spotted-fever-infected guinea pig was secured by the method of Kusama as reported by Segal (Segal 1922). The unwashed suspension from the infected animal produced spotted fever in 1/100 c. c. amounts when injected into guinea pigs. After the third washing 1 c. c. amounts of the platelet suspension failed to produce spotted fever in each of two animals. Larger quantities were not used because so small an amount of the suspension was available.

(*b*) Three cubic centimeters of serum from an infected guinea pig were then added to 3 c. c. of a platelet suspension obtained from 10 c. c. of blood of a normal guinea pig. One-tenth cubic centimeter of the unwashed mixture produced spotted fever, while 1 c. c. of platelets after the sixth washing failed to give fever when injected into each of two animals.

Experiment No. 7: Absorption of virus with fuller's earth and charcoal.—Four cubic centimeters of serum virus were added to 4 c. c. of a sterile suspension of fuller's earth (one-half gram of powdered fuller's earth in 40 c. c. of salt solution).

The mixture stood one-half hour at room temperature. Three cubic centimeters were set aside and the remaining 5 c. c. were washed 10 times by centrifugation as in previous tests upon cells. The washed and unwashed suspensions were injected into guinea pigs at the same time.

Results.—One-tenth cubic centimeter of the unwashed suspension produced spotted fever, while 1 c. c. of the washed suspension injected into each of four guinea pigs failed to give spotted fever.

The test was duplicated with the exception that finely ground charcoal was substituted for fuller's earth. The washed suspension likewise gave negative results.

DISCUSSION

In our tests we never were able to obtain an absolutely pure suspension of white cells or platelets. A few red cells could always be found when stained smears were prepared from such suspensions.

It was somewhat surprising to find that the virus was not retained by the platelets, especially since Kusama and Segal have shown that the virus of typhus fever is associated with the platelets but not with the red or white cells.

Inasmuch as the virus in the serum of animals infected with Rocky Mountain spotted fever can be thrown down only partially by high-speed centrifugation, one can not hold the view that the retention of the virus by both red and white cells (normal and infected) after slow contrifugation is due to the possession of the same specific gravity by the cells and virus. It is unlikely that the specific gravity of the virus would be such that it stratifies at the same level of both red and white cells. Furthermore, when mixed with a suspension of fuller's earth or charcoal and subjected to the same washing procedure it is not thrown down along with these particles. The test strongly suggests, therefore, that part of the virus remains lodged within or adherent to the cells.

In a cytological study of rickettsiæ in tissues of guinea pigs infected with Rocky Mountain spotted fever, Nicholson (Nicholson, 1923) says: "In some tissues it required several hours' search with the aid of a mechanical stage and in others a day or more to find them * * *. In several cases also, they appeared to be present within erythrocytes." As illustration, he gives a single figure which bears the title, "One rickettsiæ apparently within an erythrocyte."

Fricks (Fricks, 1916a) has described, in the centrifuged red blood cells of infected animals, "round or slightly elongated red chromatin bodies partially surrounded by or in close approximation to a somewhat larger deep-blue staining body." Such bodies were never found in control specimens. Fricks suggested that these bodies were of protozoan nature, but hesitated to draw definite conclusions from his findings.

We are inclined to regard as highly significant the fact that, in the examination of hundreds of specimens of infected blood, rickettsiæ could not be demonstrated by us within erythrocytes, and yet the infective agent could not be washed or diluted away from such cells.

The difficulty with which rickettsiæ are demonstrated microscopically in tissues of infected animals is not compatible with the general intense plasmatic and systemic infection as shown by animal inoculation, although we realize the possibility that a single organism or very few organisms may be infective. It seems likely, however, that the few rickettsiæ (*Dermacentroxenus rickettsi* Wolbach) found represent, in part, one phase of the infective agent, but they do not convincingly represent all known manifestations of the blood virus. In this connection it may be stated that one receives the same impression when a study is made of the presence and distribution of rickettsiæ in the tissues of infected ticks.

Our tests might be regarded as suggesting that the virus of Rocky Mountain spotted fever can assume in the mammalian host a phase which can not be demonstrated to the eye by methods thus far used.

SUMMARY

1. The serum from guinea pigs infected with Rocky Mountain spotted fever and subjected to high speed (8,800 revolutions per minute) centrifugation retains infectivity in the top portion.

2. After repeated washings by slow speed (1,200 revolutions per minute) centrifugation, red and white blood cells from infected guinea pigs are capable of transmitting the infection, although the demonstration of organisms in fresh preparations or stained smears of such cells is extremely rare.

3. Normal red and white blood cells to which serum virus has been added retain the infection after repeated washings by centrifugation.

4. A suspension of platelets from an infected guinea pig, as well as a suspension from a normal animal to which virus has been added, does not retain the infection after washing by centrifugation.

5. Suspensions of fuller's earth or charcoal, to which serum virus has been added, quickly lose infectiousness when washed by centrifugation.

6. The suggestion is made that the virus of Rocky Mountain spotted fever may assume a form incapable of demonstration by known methods.

VARIATIONS IN THE BEHAVIOR OF THE VIRUS

By R. R. Spencer, Surgeon, and R. R. Parker, Special Expert, United States Public Health Service

Rocky Mountain spotted fever belongs to the group of human infections transmitted by the bites of certain blood sucking insects and arachnids which function as true biological hosts. The causative organism, therefore, must adapt itself during one period of its life history to the environmental conditions within the warm blooded mammalian host, and during another to the entirely different conditions within the cold-blooded arthropod. It is then reasonable to assume that, as in malaria, trypanosomiasis, filariasis, and other diseases of this group, this change in environment is accompanied by morphological and functional changes in the disease agent. In Rocky Mountain spotted fever, however, as well as in other diseases associated with rickettsiæ (typhus and trench fever), such changes have not been satisfactorily demonstrated.

In a previous paper (see p. 22) we have suggested the occurrence of three phases in the life history of the virus. Further studies have supported the existence of these phases, which are here described.

1. A noninfectious phase in hibernating fasting ticks which is not capable of producing the typical disease unless stimulated by blood or heat but which will frequently immunize when injected into guinea pigs.

2. A highly infectious and virulent phase in nymphs and adults following feeding. This phase in adult ticks which have been infected in an earlier stage is usually accompanied by a marked increase in rickettsiæ throughout the tick tissues, but in the same phase in adults recently infected as adults, rickettsiæ are not numerous and may be absent despite a high concentration of live virus. It is also this phase of the virus in fed adults with or without rickettsiæ from which a protective vaccine can best be prepared.

3. A mammalian blood or tissue-virus phase which lacks the penetrating power or invasiveness of tick virus and which rarely possesses protective quality when treated with phenol.

The results recorded in Table 21 demonstrate the two stages of the virus in the tick; namely, the hibernating noninfectious immunizing stage, and the virulent infectious stage.

TABLE 21.—*Infectivity of virus content of hibernating and fed adult ticks—lot 4054–(2aA) infected as larvæ*

HIBERNATING TICKS

Test No.	Method	Result
A 1	One guinea-pig received pooled viscera of 10 ticks intraperitoneally.	No symptoms in 16 days. Subsequently immune.
A 2	----do----	Do.

TICKS INCUBATED FOUR DAYS AT 37° C.

Test No.	Method	Titration of pooled virus							
		One tick	1/10	1/100	1/500	1/1,000	1/5,000	1/10,000	1/15,000
B3	A series of 16 guinea pigs received graded dilutions of pooled ground viscera of 10 ticks intraperitoneally----	S. F. +	S. F.	S. F.	S. F.	–	–	–	–

TICKS FED FOUR DAYS

C4	A series of 16 guinea pigs received graded dilutions of pooled ground viscera of 10 ticks intraperitoneally----	S. F. +	S. F. +	S. F. +	S. F. +	S. F. +	–	S. F. +	–

Symbols.—S. F. = spotted fever; + = died; – = negative.

Ticks that were infected as larvæ and reared to adult stage were placed out of doors in glass cylinders on March 1, 1926. The following midwinter (Jan. 20, 1927) 40 individuals of this lot were brought indoors and 2 guinea pigs immediately inoculated intraperitoneally with the ground viscera of 10 ticks, each suspended in salt solution (A). Neither of these pigs developed symptoms, al-

though both showed a slight elevation of temperature for a few days. Both animals were immune to 1 c. c. of guinea-pig blood virus given on the sixteenth day.

Ten other ticks (B) were incubated (37° C.) for four days, then the pooled viscera ground and suspended in salt solution. A series of guinea pigs was injected with graded dilutions of this suspension. Guinea pigs receiving the virus equivalent of one whole tick died. Pigs receiving less amounts of virus down to 1/500 dilution developed spotted fever but recovered.

The remaining 10 ticks were fed four days (C), then a pooled suspension made in the same manner and another series of animals inoculated with graded dilutions. It will be noted that the feeding increased the minimal infectious dose per tick to a greater extent than did incubation. This was to be expected, considering the ingestion of blood and the subsequent active growth of the tick. It should be pointed out that while only the animals receiving the virus equivalent of one whole tick from the incubated group (B) died, among the animals receiving the virus from the fed ticks (C), all died that developed the fever, even though the infecting dose was as low as 1/10,000 of a tick. Results of this kind suggest an increased virulence as well as an increase in the number of the minimal infectious doses following tick feeding.

The third, or mammalian blood phase of the virus, we believe to be qualitatively distinct from the above-described tick phases for the following reasons:

First, the blood virus does not possess the penetrating power of tick virus as shown in another paper (p. 60). The tick virus was shown to possess the power to penetrate the unbroken skin, the conjunctiva, and the mucous membrane of the alimentary track. In no instance did the blood virus do this. This quality could not be explained satisfactorily by assuming a greater concentration of the tick virus since it was diluted to the approximate concentration of the undiluted blood virus used in the same test.

Secondly, a prolonged incubation period follows as a rule the injection into guinea pigs of minimal infectious doses, or small multiples of this dose, of either blood or tick virus. However, when very large doses of blood virus (5,000 to 10,000 M. I. D., i. e., 5 to 10 c. c. of whole blood) are given the incubation period is not shorter nor the infection severer than that produced by ½ c. c. On the other hand, identical large doses of tick virus (5,000 to 10,000 M. I. D., i. e., the viscera of one tick) may completely overwhelm the animals and cause death so early that the typical symptoms of the disease—namely, fever, testicular swelling, and enlarged spleen—fail to develop.

Finally, in contradistinction to tick virus, it is extremely rare that animals can be immunized by the injection of large amounts of tissue or blood virus treated with phenol, though repeated injections are given. Connor (Connor, 1924 *b*) has treated the tissue virus with various chemicals and injected the material in large quantities without producing an immunity. We have found the virus in the testicles of a rabbit to yield 30,000 M. I. D. per gram of tissue; yet, phenolized suspensions of the same tissue failed to immunize. Occasionally we have had the killed tissue virus produce an immunity of moderate degree, but our results were very inconstant. The tick virus, on the other hand, rarely fails to immunize, and we have found a single injection of only 1/20 c. c. of phenolized tick virus, representing 250 M. I. D. to protect guinea pigs.

INCUBATION PERIOD OR PERIOD OF INVASION IN THE TICK

When normal adult ticks are infected by feeding on an infected guinea pig, they are not capable of transmitting the infection by feeding for the first nine days at least. This incubation period in the tick corresponds perhaps to the period in which the virus is invading the various tissues of the tick and ends when it finally reaches the salivary gland or mouth parts, assuming infection normally occurs in this manner.[18] Although during this incubation period the ticks are incapable of transmitting infection by feeding, the virus may usually be demonstrated (but not invariably) by injecting ground suspensions of the ticks into susceptible guinea pigs. Furthermore, the virus content of such ticks may be greatly increased by a second feeding during the incubation period without the tick infecting its host.

In Table 22, this increase in virus content, the period of incubation, and the protective value of vaccine made from such virus is shown. It may be seen that the pooled-viscera suspension of the first group of 11 ticks which were tested immediately after the first or infectious feeding produced infection only in 1/10 dilutions, and only 1 of 10 pigs receiving vaccine prepared from the same material was protected.

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[18] It is interesting to note that this period is also about the same length as the so-called extrinsic incubation in yellow fever, which was first called attention to by Carter, and recently demonstrated in animals by Bauer and Hudson (Jour. Exp. Med., July 1, 1928) in the mosquito, *Aedes aegypti*.

TABLE 22.—*Results of second feeding of adult ticks at increasing intervals after infective feeding—duration of infective feeding, three days; duration of second feeding, three days*

Number of ticks fed	Interval between first and second	Result of second feeding of ticks on a single guinea pig	Titration of pooled virus from same ticks after second feeding							Protective value of vaccine prepared from the same virus									
										Guinea pigs Nos.									
			1/10	1/100	1/500	1/1,000	1/5,000	1/10,000	1/15,000	1	2	3	4	5	6	7	8	9	10
11	No second feeding titrated immediately after first feeding		S. F.	—	—	—	—	—	—	—	—	—	+	V.	—	V.	—	V.	—
11	3 days	Negative	S. F.	S. F.	S. F.	—	—	—	—	V.	+	—	—	—	—	—	+	P.	V.
11	6 days	do	S. F.	S. F.	S. F.	—	—	—	—	V.	+	+	P.	+	+	+	+	P.	P.
11	9 days	do	S. F.	S. F.	—	S. F.	—	S. F.	—	+	P.	+	V.	+	V.	+	+	+	+
11	12 days	Spotted fever	S. F.	S. F.	S. F.	S. F.	S. F.	S. F.	—	+	V.	+	+	+	+	V.	V.	+	+

Symbols.—S. F. = spotted fever; P. = partial protection; V. = valueless; — = no spotted fever or no protection; + = full protection.

The second group of 11 ticks which received a second feeding beginning three days after the infectious feeding produced infection in dilutions of 1/500, and the vaccine prepared from the same virus immunized 2 of 10 animals.

The third group of ticks, fed after an interval of six days, gave approximately the same amount of virus, but 6 of the 10 animals receiving the vaccine were immune.

The fourth group, feeding after nine days, showed a much higher virus content (10,000 M. I. D. per tick) and protected 7 of 10 animals. None of these four groups of ticks infected the guinea pigs on which they fed, although all contained virus.

The fifth group of 11 ticks, feeding on the twelfth day, *infected the animal on which they fed*, possessed 10,000 doses per tick and the vaccine gave good protection.

From the results of tick feeding it is seen that the incubation period, or the time required for the tick to become infectious by feeding, lies between the ninth and twelfth day.

In Table 23 the results of an experiment are shown in which ticks were fed continuously for 10 days immediately after the infectious feeding, permitting each tick to feed throughout this period upon the same guinea pig until full engorgement was reached. It will be noted that only 3 (Nos. 4, 9, and 10), of the 10 ticks, infected the guinea pigs upon which they fed and that in 5 (Nos. 3, 5, 6, 7, and 8) no rickettsiæ were seen in the smears of any of their organs. Neverthlesss, the pooled ground viscera that remained after smears were made of all 10 ticks gave infection in dilutions of 1/15,000 part of a tick. Perhaps even higher dilutions would have been infectious, but they were not tried.

TABLE 23.—*Result of 10 days' continuous feeding of 10 adult ticks (infected as adults) each on 10 normal guinea pigs immediately after infective feeding*

Tick and guinea pig No	Result of 10 days' feeding on normal guinea pigs	Smears of same 10 ticks						Titration of pooled virus of same ticks						
		S. G.	Br.	Gut	M. T.	M.	R. O.	1/10	1/100	1/500	1/1,000	1/5,000	1/10,000	1/15,000
1	Negative	−	−	+	+	−	+	S. F.	S. F.	S. F.	S. F.	S. F.	S. F.	S. F.
2	do	−	+	+	+	+	+	All ticks fed to full engorgement.						
3	do	−	−	−	−	−	−							
4	Spotted fever—onset 14 days after beginning of feeding	+	+	+	+	+	+							
5	Negative	−	−	−	−	−	−							
6	do	−	−	−	−	−	−							
7	do	−	−	−	−	−	−							
8	do	−	−	−	−	−	−							
9	Spotted fever—onset 10 days after beginning of feeding	−	+	−	−	+	−							
10	do	+	+	+	−	+	+							

Symbols.—S. F.=spotted fever; + = rickettsiæ; S. G. = salivary gland; Br.=brain; Gut = intestines; M. T. = Malpighian tubules; M. = Muscle; R. O.= reproductive organs.

A period of 10 days or longer elapsed after the beginning of tick feeding before guinea pigs Nos. 4, 9, and 10 developed fever. This would suggest that the ticks did not infect the animals during the first days of feeding, or, in other words, during the period of virus invasion, since ordinarily guinea pigs will develop symptoms within four or five days after tick feeding.

THE RÔLE OF RICKETTIÆ

Our conception of the above-described phases of the virus of Rocky Mountain spotted fever is seen to be based entirely upon the functional behavior of the virus and not upon morphological characteristics of the associated rickettsiæ. We were disappointed when extensive studies upon rickettsiæ in infected ticks, controlled by similar studies of noninfected ticks, failed in our hands to reveal a clear-cut morphological sequence or to reveal any forms that could be considered as of diagnostic value. From morphological characters alone, *Dermacentroxenus rickettsi*, described by Wolbach (Wolbach 1919–b) as the causative agent of Rocky Mountain spotted fever, could well be included in the genus Rickettsia, if we accept Cowdry's (Cowdry, 1926) definition.[19] However, Wolbach restricts the term "rickettsia" to microorganisms adapted to arthropods and pathogenic for vertebrates.

Wolbach found *D. rickettsi* in ticks of proved infectivity and in tissues of human cases. He described three morphological types, and was unable to find similar forms in proved noninfective ticks.

On the whole, our observations support those of Wolbach in that rickettsiæ can always be found at one stage or another in the life cycle of ticks known to be infected. (See pls. 4 to 9.) But they are not seen at every stage, and our observations suggest that the recognized forms alone do not represent all phases of the virus. We have also shown (p. 36) that we can not depend upon the mere presence or absence of rickettsiæ in smears of ticks as a criterion of infectiousness, since identical forms have been seen by us in known noninfected ticks. However, it has been amply demonstrated that as soon as fasting adult ticks, infected in an early stage, are fed, one can readily find, either by smears or sections, in the majority of such ticks, but not in all, myriads of rickettsiæ throughout the tick tissues. This increase coincides fairly closely with the increase in the amount of infective virus in the tick viscera. Yet, if some of these ticks remain for a month or longer at room temperature the virus content will gradually decrease, and, in some ticks, disappear entirely (although

[19] Cowdry defines rickettsiæ as "gram-negative bacteriumlike organisms of small size usually less than half a micron in diameter which are found intracellularly in arthropods, which may be more or less pleomorphic and stain rather lightly with analine dyes, but which resemble in most of their properties the type species *R. prowazeki*."

the power to immunize will usually be retained); but the same ticks reveal in smears typical rickettsiæ as numerous as those seen in ticks of the same group just following feeding and containing 10,000 doses of live virus per tick.

In striking contrast to this picture of numerous rickettsiæ with slight or no infectiousness, is that seen in smears of refed recently infected adult ticks. In such ticks it was a great surprise to find that the increase in infectiousness was not accompanied by any marked increase in rickettsiæ. In fact, many series of these ticks were smeared and stained, finding only a few typical rickettsiæ, although suspensions made from the same ticks contained 10,000 M. I. D. per tick. These observations give the impression that the infectious stage of the virus is represented by forms that are so minute as to be beyond the range of the ordinary microscope or are not easily stained by our present methods. The rickettsiæ found after feeding of adults infected as larvae are usually quite small at first, and occur in coccoidal, lanceolate, and diplobacillary forms, while those found in ticks from the same lots that have remained at room temperature several weeks or longer are larger and distinctly bacillary in form, and it would be difficult to distinguish them from ordinary bacteria. Filiform organisms also frequently develop.

On the other hand, in ticks containing large quantities of active virus with few rickettsial forms we often see numerous fine red staining granules which are difficult to distinguish from granules seen in normal ticks, but which give the impression of a prerickettsial stage.

In this connection, it is interesting to compare the work of Manouelian and Viala (Manouelian and Viala, 1926) on rabies. These investigators have shown that in street rabies there exists no relation between the abundance of Negri bodies and the clinical forms of the disease, and that an exaltation of the rabies virus is accompanied by the disappearance of Negri bodies. They find only a very minute form, *Encephalitozoon rabiei*, as a constant accompaniment of all forms of rabies.

In ticks infected with Rocky Mountain spotted fever we have encountered no microscopic forms of any kind which may be said to be a constant accompaniment of the infection. The presence of virus in ticks from nature can be verified or ruled out only by feeding the ticks and subsequently injecting the ground viscera into susceptible animals, which must later be given an immunity test if infection does not follow either tick feeding or the injection of the tick viscera.

Some specimens have been encountered in which the morphology, abundance, and arrangement of the rickettsiæ were typical in every way and yet the ticks would not infect the guinea pigs on which they fed or those into which the tick viscera were subsequently injected.

TABLE 24.—*Comparison of adult ticks infected as larvæ with adults infected as adults—as to presence of rickettsiæ—amount of virus and protective power of vaccine*

INFECTED AS LARVÆ—11 TICKS USED IN EACH TEST

Time of test in relation to feeding	Tick smears												Titration of pooled virus from same ticks							Protective value of vaccine prepared from same ticks. Guinea pigs Nos.									
	Tick tissue	1	2	3	4	5	6	7	8	9	10	11	1/10	1/100	1/500	1/1000	1/5000	1/10,000	1/15,000	1	2	3	4	5	6	7	8	9	10
2 days before adult feeding	S. G.	−	−	+	+	−	+	−	+	+	−	+	−	−	−	−	−	−	−	I.	I.	I.	I.	I.	I.	I.	I.	I.	V.
	Br.	+	+	+	+	−	+	−	+	+	+	+																	
	Gut.	−	+	+	+	+	−	+	−	+	+	+																	
	M. T.	+	+	+	+	+	+	+	+	+	+	+																	
	M.	+	+	+	+	+	+	−	+	+	+	+																	
	R. O.	+	+	+	+	+	+	+	+	+	+	+																	
Immediately after feeding	S. G.	+	+	+	+	−	−	−	+	+	+	+	S. F.	S. F.	S. F.	S. F.	S. F.	−	−	I.	I.	I.	I.	I.	I.	P.	I.	P.	I.
	Br.	+	+	+	+	+	+	+	+	+	+	+																	
	Gut.	−	+	−	+	−	−	−	−	−	+	−																	
	M. T.	+	+	+	+	−	−	−	+	+	+	+																	
	M.	+	+	+	+	+	+	+	+	+	+	+																	
	R. O.	+	+	−	−	−	−	−	+	+	+	+																	
2 days after feeding	S. G.	+	+	+	+	+	+	+	+	+	+	+	S. F.	S. F.	−	S. F.	−	−	−	V.	V.	I.	I.	I.	I.	I.	I.	P.	I.
	Br.	+	+	+	+	+	+	+	−	+	+	+																	
	Gut.	+	+	+	+	+	+	+	−	+	+	+																	
	M. T.	+	+	+	+	+	+	+	+	+	+	+																	
	M.	+	+	+	+	+	+	+	−	+	+	+																	
	R. O.	+	+	+	+	+	+	+	−	+	+	+																	
4 days after feeding	S. G.	+	+	+	+	+	+	+	−	−	+	−	S. F.	S. F.	S. F.	S. F.	−	−	−	P.	I.	I.	I.	P.	I.	P.	P.	P.	I.
	Br.	+	+	+	+	+	+	+	−	−	+	−																	
	Gut.	−	+	−	−	−	+	+	+	−	−	+																	
	M. T.	+	+	+	+	+	+	+	−	−	+	+																	
	M.	+	+	+	+	+	+	+	+	+	+	−																	
	R. O.	+	+	+	+	+	+	+	−	−	+	−																	

INFECTED AS ADULTS—11 TICKS USED IN EACH TEST

Immediately after first or infecting feeding	S. G.	−	−	−	−	−	−	−	−	−	−	−	S. F.	−	−	−	−	−	−	−	−	−	I.	V.	−	V.	−	V.	I.
	Br.	−	−	−	−	−	−	−	−	−	−	−																		
	Gut.	−	−	−	−	−	−	−	−	−	−	−																		
	M. T.	−	−	−	−	−	−	−	−	−	−	−																		
	M.	−	−	−	−	−	−	−	−	−	−	−																		
	R. O.	−	−	−	−	−	−	−	−	−	−	+																		
Second feeding begun 3 days after infecting feeding	S. G.	−	−	−	−	−	−	−	−	−	−	−	S. F.	S. F.	S. F.	−	−	−	−	V.	I.	−	−	−	−	−	I.	P.	V.
	Br.	−	−	+	−	−	−	−	−	−	−	−																		
	Gut.	−	−	−	−	−	−	−	−	−	−	−																		
	M. T.	−	−	−	−	−	−	−	−	−	−	−																		
	M.	−	−	+	−	−	−	−	−	−	−	−																		
	R. O.	−	−	−	−	−	+	−	−	−	−	−																		
Second feeding begun 6 days after infecting feeding	S. G.	−	+	−	−	−	−	−	−	−	−	−	S. F.	S. F.	S. F.	−	−	−	−	V.	I.	I.	P.	I.	I.	I.	I.	P.	P.
	Br.	−	+	−	−	−	−	−	−	−	−	−																		
	Gut.	−	+	−	−	−	−	−	−	−	−	−																		
	M. T.	−	+	−	−	−	−	−	−	−	−	−																		
	M.	−	+	−	−	−	−	−	−	−	−	−																		
	R. O.	−	+	−	−	−	+	+	+	−	−	−																		
Second feeding begun 9 days after infecting feeding	S. G.	−	−	−	−	−	−	−	−	−	−	−	S. F.	S. F.	−	S. F.	−	S. F.	−	I.	P.	I.	V.	I.	V.	I.	I.	I.	I.
	Br.	−	+	−	+	−	−	−	−	−	−	−																		
	Gut.	−	+	−	−	+	−	−	−	+	−	−																		
	M. T.	−	−	−	−	−	−	−	−	−	−	−																		
	M.	−	−	−	−	−	−	−	−	+	−	−																		
	R. O.	+	+	−	+	−	+	+	+	−	−	+																		
Second feeding begun 12 days after infecting feeding	S. G.	−	−	−	−	−	−	−	+	−	−	−	S. F.	S. F.	S. F.	S. F.	S. F.	S. F.	−	I.	V.	I.	I.	I.	I.	V.	V.	I.	I.
	Br.	−	+	−	−	−	−	−	+	−	−	−																		
	Gut.	+	−	−	−	−	−	−	+	−	−	−																		
	M. T.	−	−	−	+	−	−	−	+	+	−	−																		
	M.	−	−	−	−	−	−	−	+	−	−	−																		
	R. O.	−	−	−	−	−	−	−	+	−	−	−																		

Symbols.—S. F. = spotted fever; + = rickettsiæ; S. G. = salivary gland; Br = brain; Gut = intestines; M. T. = malpighian tubules; M. = muscles; R. O. = reproductive organs; I. = immune; P. = partial immunity; V. = valueless; − = negative.

In Table 24 is shown the striking difference in the presence of rickettsiæ in the various organs of adult ticks infected as larvæ with the presence of rickettsiæ in the organs of adults recently infected as adults. Even where rickettsiæ are found in the recently infected adults they were always less numerous than in the smears of the larvæ-infected adults. This relative abundance could not be represented in the table. In recently infected adults it should also be noted that although the number of minimal infectious dose per tick was large, and the immunizing quality of the virus excellent, the rickettsiæ were nevertheless infrequent in the organs. On the other hand, the organs of the larvæ-infected adults, before feeding, contained many rickettsiæ but no active virus, according to the titration of their pooled viscera. The virus was present therefore in a dormant phase only in this first group of 11 ticks tested before feeding, but, nevertheless, it possessed high protective value when prepared into a vaccine, as shown by the fact that 9 out of 10 guinea pigs were thus protected.

INFECTION BY OTHER MEANS THAN TICK BITES

By R. R. Spencer, Surgeon, and R. R. Parker, Special Expert, United States Public Health Service

No definitely proven case of Rocky Mountain spotted fever has ever been reported as originating in an area where wood ticks, *Dermacentor andersoni* Stiles, were not prevalent. Not infrequently, however, cases of natural infection have occurred without proof of tick bite, and among those contracting the infection in the laboratory a history of tick bite has been the exception rather than the rule. Since 1912, 14 laboratory workers have contracted the disease, 9 of whom gave neither history nor other evidence of tick bite.

Other means by which the virus may gain access to the body of the susceptible host is indicated by experiments summarized in the following table.

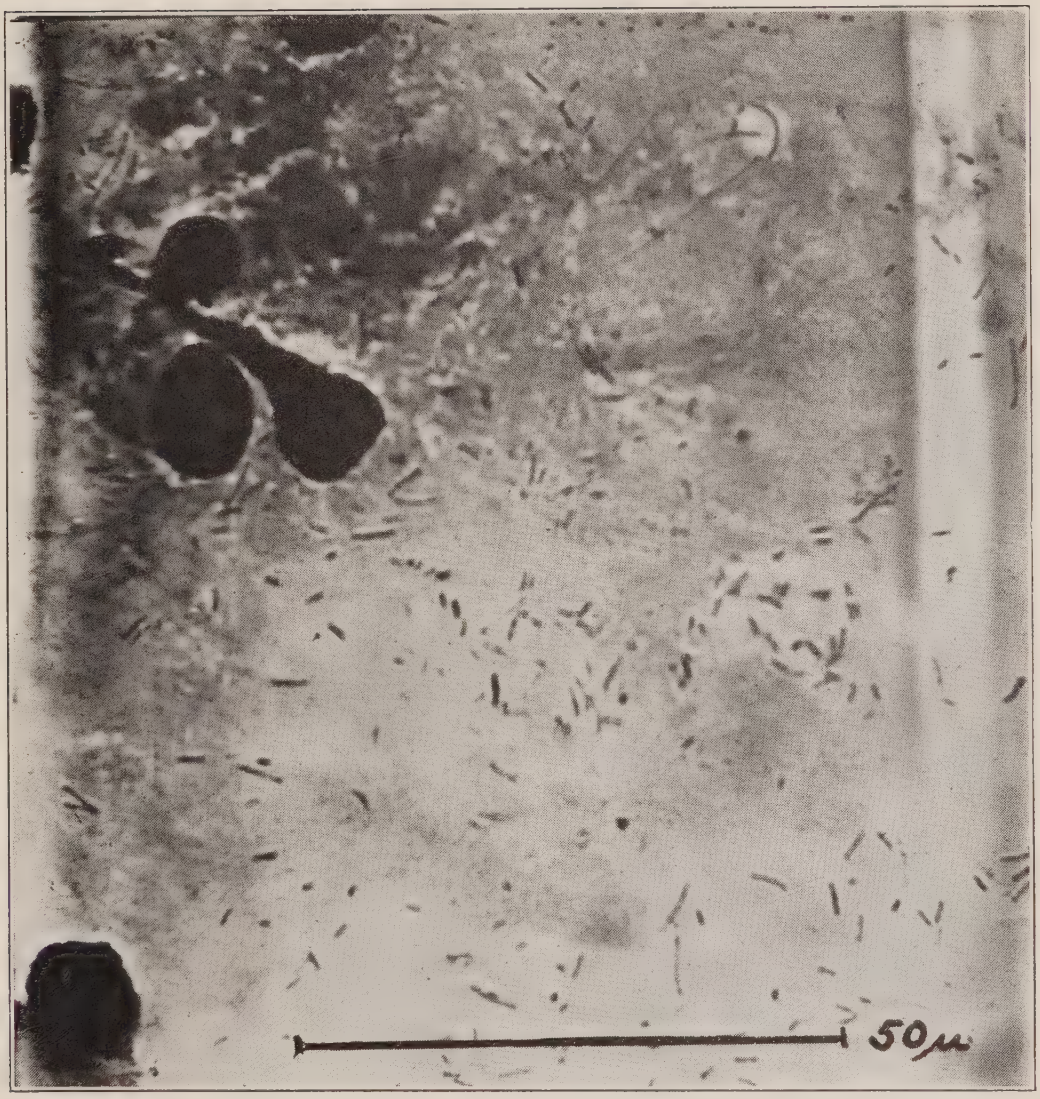


Plate 4.—Photomicrograph of Giemsa-stained smear of the Malpighian tubules of an infected fed adult tick. Rickettsiaelike organisms associated with the disease are abundant in fed infected ticks and approach the size of ordinary bacteria

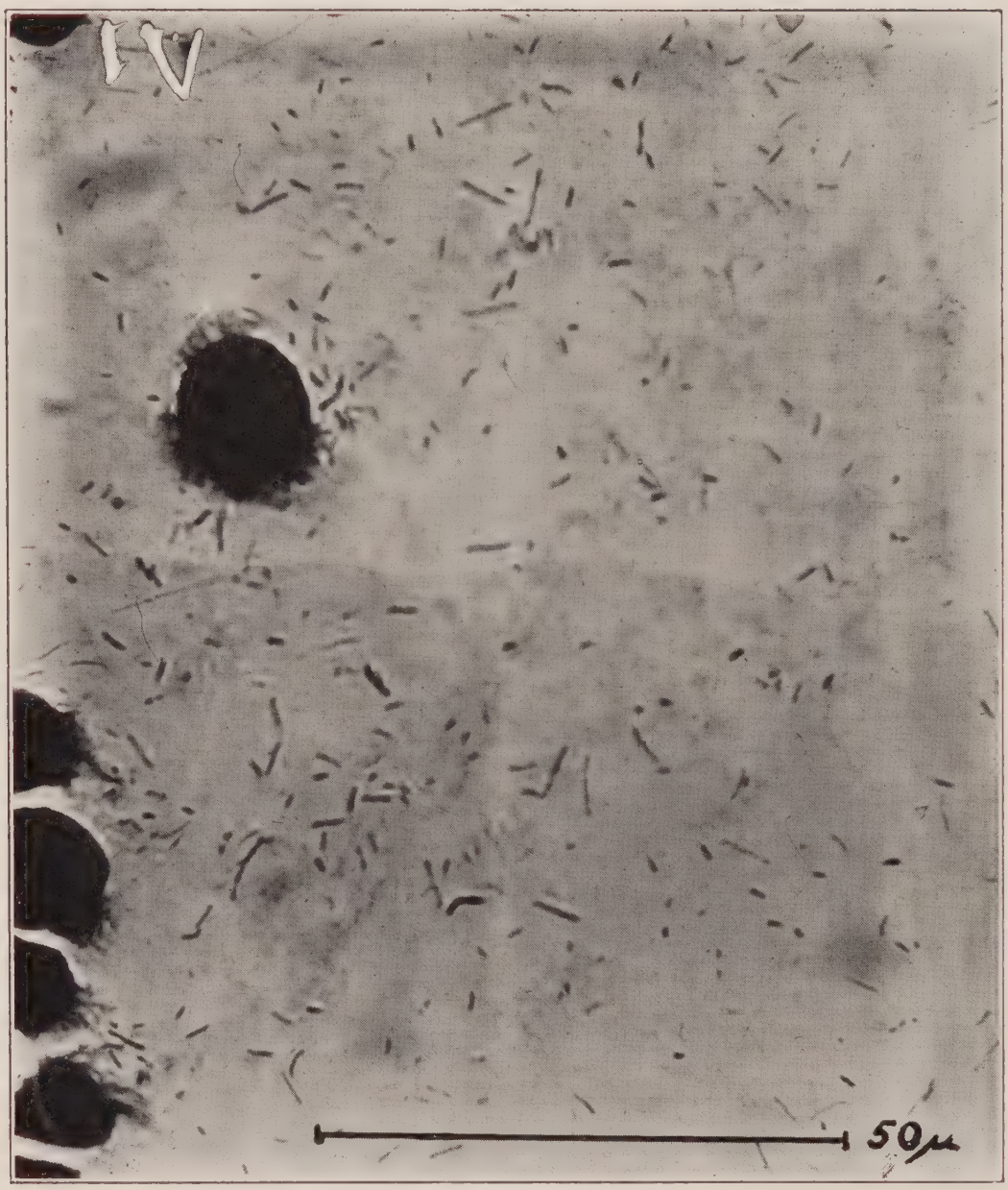


Plate 5.—Photomicrograph similar to Plate 4

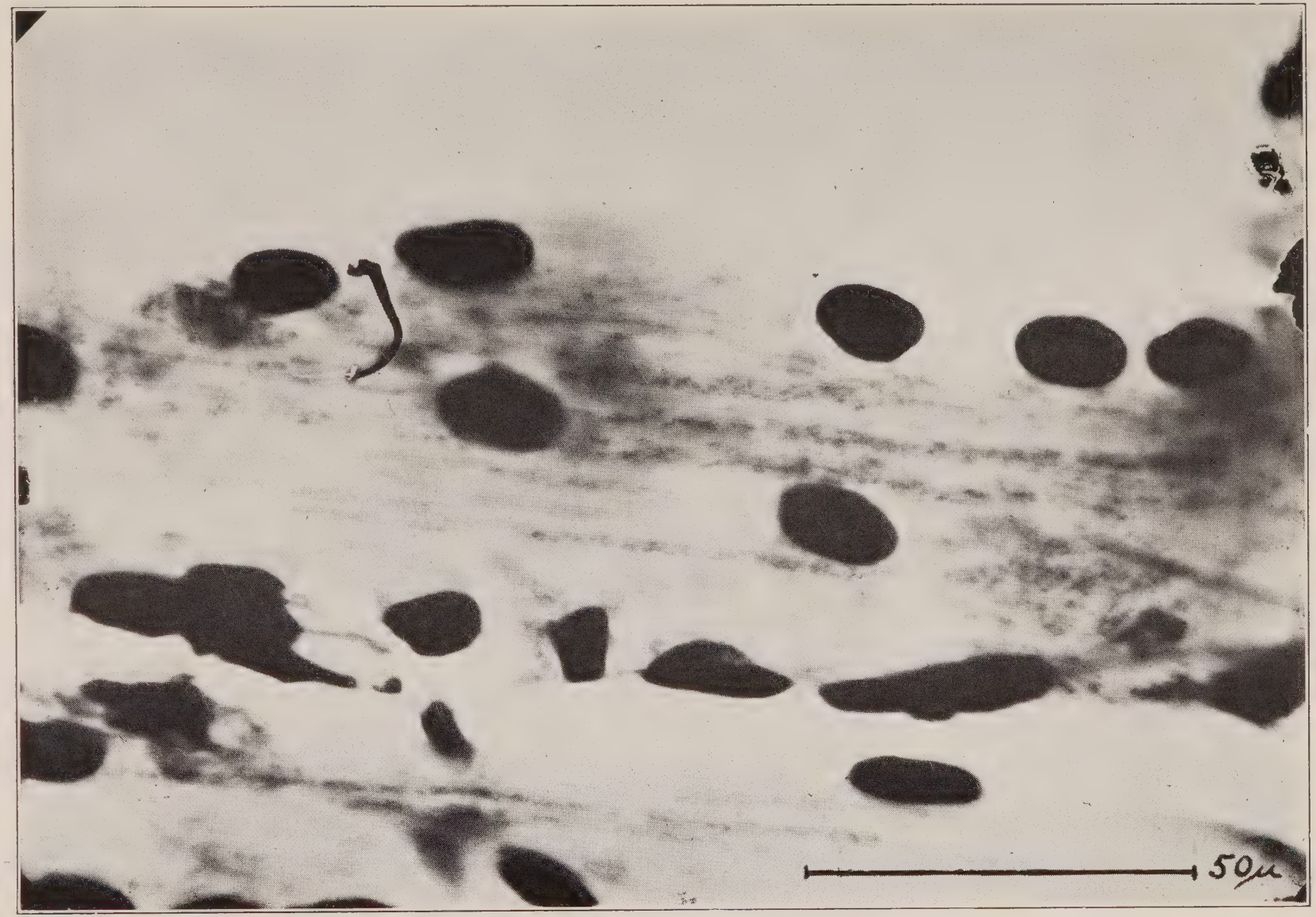


Plate 6.—Photomicrograph of the sucking muscles of an infected tick showing the organisms lying between the muscle fibers in linear masses

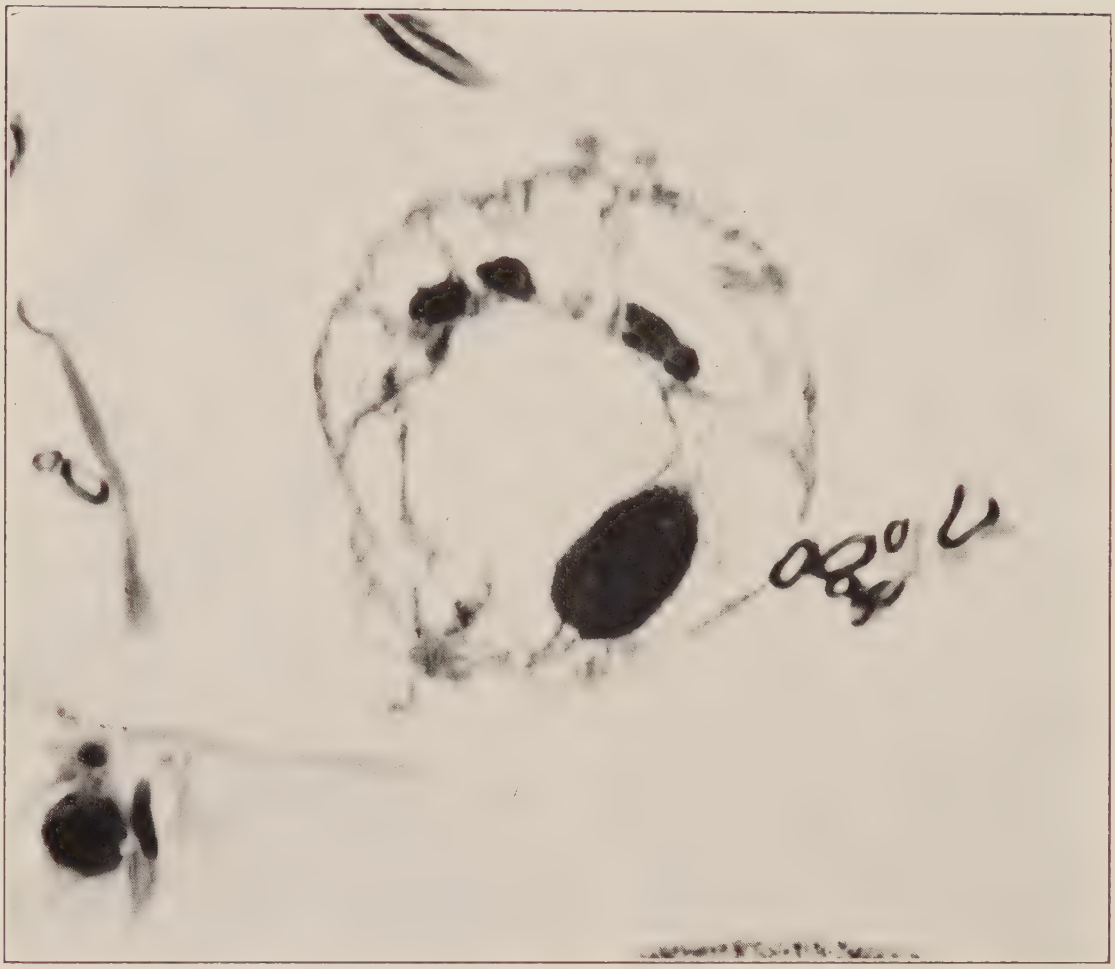
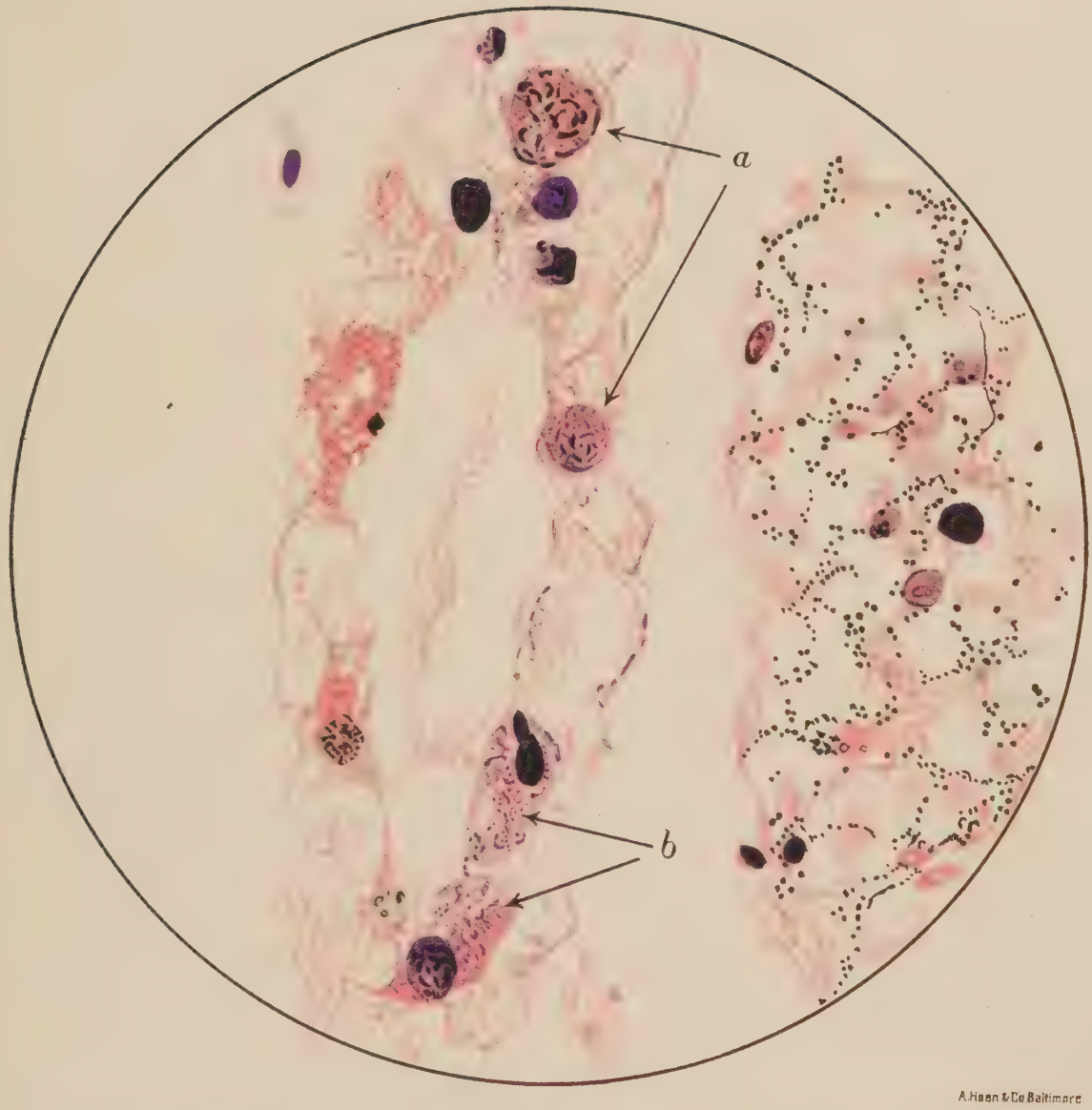


Plate 7.—Cross section of the Malpighian tubule of an infected hibernating unfed tick showing a nucleus greatly distended with very small forms. After tick feeding, intranuclear forms have not been encountered

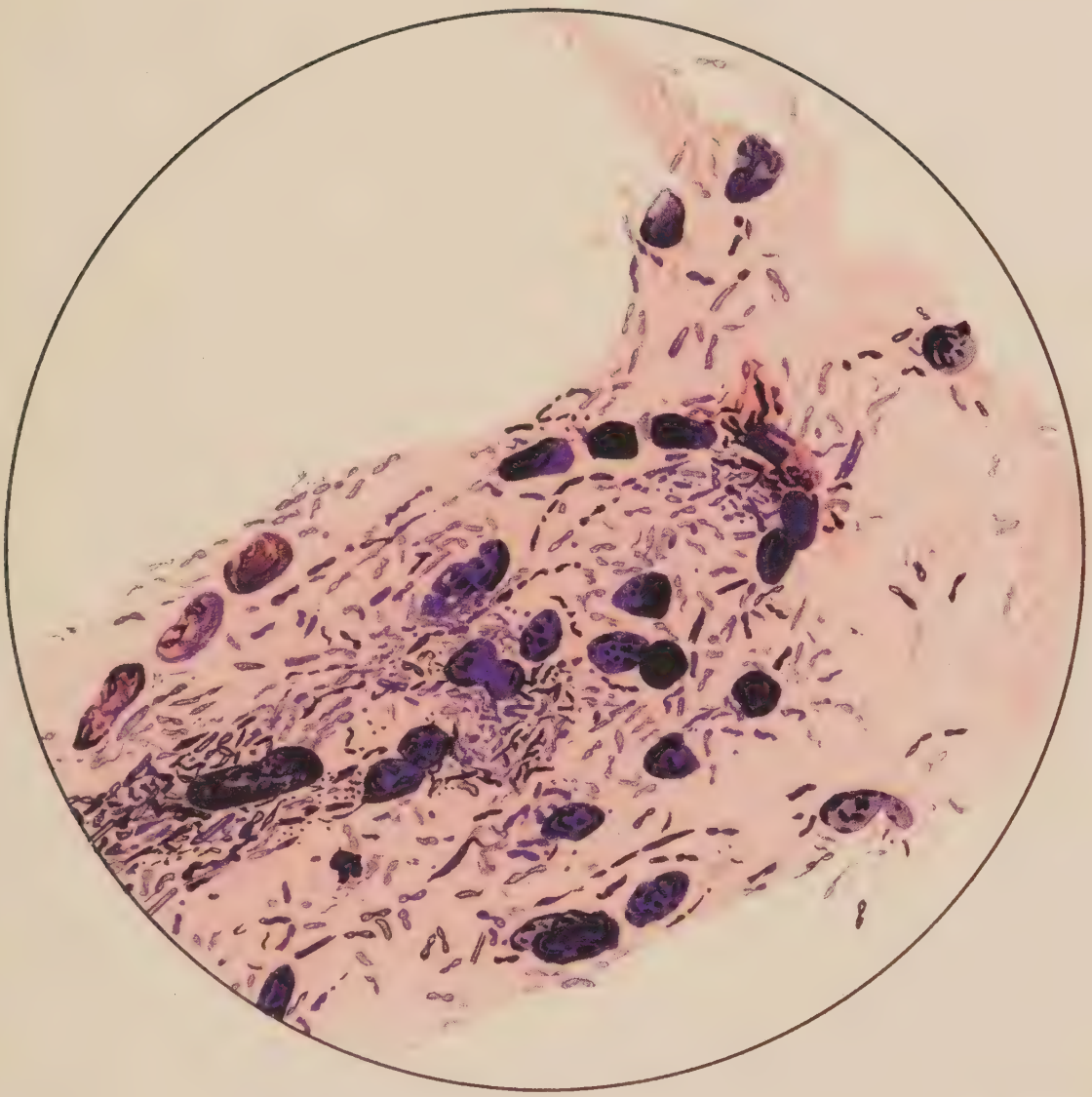
PLATE 8



A.Hoen & Co.Baltimore

Longitudinal section of the Malpighian tubule of an infected, not fed, hibernating adult tick. Nuclei are seen distended with rickettsiae at (*a*). Intracellular forms at (*b*)

PLATE 9



A. Hoen & Co. Baltimore.

Camera lucida drawing of the rickettsiae in a piece of tick muscle. Teased smear preparation; Giemsa stained. The tick was proved infected by guinea pig inoculation

TABLE 25.—*Comparison of penetrating power of blood and tick virus*

BLOOD VIRUS

G. P. No.	Virus in conjunctival sack	G. P. No.	Virus in mouth	G. P. No.	Virus on normal skin	G. P. No.	Virus on epilated skin	G. P. No.	Virus on shaved skin
1	Died of pneumonia	21	Negative 13 days	41	Negative 13 days	61	Negative 13 days	81	Negative 13 days.
2	do	22	do	42	do	62	do	82	Do.
3	Negative 13 days	23	do	43	do	63	do	83	Do.
4	do	24	do	44	do	64	do	84	Do.
5	do	25	do	45	do	65	Died, secondary infection	85	Do.
6	Died of pneumonia	26	do	46	do	66	Negative 13 days	86	Died of pneumonia.
7	Negative 13 days	27	do	47	do	67	do	87	Negative 13 days.
8	do	28	do	48	do	68	do	88	Do.
9	do	29	do	49	do	69	Died of pneumonia	89	Do.
10	do	30	do	50	do	70	Negative 13 days	90	Do.
	0 per cent.		0 per cent.		0 per cent.		0 per cent.		0 per cent.

TICK VIRUS

11	Negative 13 days	31	Negative 13 days	51	Negative 13 days	71	Spotted fever	91	Spotted fever.
12	Spotted fever	32	do	52	Spotted fever	72	Negative 13 days	92	Do.
13	Negative 13 days	33	do	53	do	73	Spotted fever	93	Do.
14	Spotted fever	34	Spotted fever	54	Negative 13 days	74	do	94	Do.
15	Negative 13 days	35	Negative 13 days	55	Spotted fever	75	do	95	Do.
16	Spotted fever	36	do	56	do	76	do	96	Do.
17	Negative 13 days	37	do	57	Negative 13 days	77	do	97	Do.
18	Spotted fever	38	Spotted fever	58	do	78	Negative 13 days	98	Do.
19	do	39	Negative 13 days	59	Spotted fever	79	do	99	Do.
20	Negative 13 days	40	do	60	Negative 13 days	80	Spotted fever	100	Do.
	50 per cent.		20 per cent.		50 per cent.		70 per cent.		100 per cent.

Explanation of table.—One hundred guinea pigs were used in the test. Half of these were treated with blood virus, and half with tick virus, employing identical technic in both series. For one series, citrated blood undiluted from an infected guinea pig at the height of the fever was employed as the infecting material. For the other series, infected tick tissue suspension was made up so that each cubic centimeter contained the virus equivalent of one tick, and then diluted 10 times. The tick suspension was thus diluted so that the minimal infectious dose per cubic centimeter would approximate that in the citrated whole blood. The blood of infected animals does not contain more than 1,000 infectious doses per cubic centimeter, while infected tick tissue suspension (1 tick per cubic centimeter) rarely contains more than 10,000 doses per cubic centimeter.

A single drop of the citrated undiluted blood virus was now placed in the conjunctival sack of the left eye of each of 10 guinea pigs. Four other groups of 10 pigs each were treated by placing one drop in the mouth, by placing it on the skin after removing the hair with the fingers, and finally by placing it on the shaved skin.

The remaining 50 guinea pigs were treated in groups of 10 each in the same manner with the exception that one drop of the diluted tick virus suspension was substituted for the undiluted blood virus.

It may be seen that in no instance did the blood virus infect the animals, although control animals (not shown in the table) injected subcutaneously with the same virus suspension developed the disease.

On the other hand, the tick virus suspension infected all 10 of those receiving it on the shaved skin; 7 of those receiving it on the epilated skin; 5 receiving it in the conjunctival sac, 5 receiving it on the normal skin, and 2 receiving it in the buccal cavity.

Discussion.—These results indicate that tick virus has a penetrating power that blood virus does not possess.

In considering the possibility of human infection by other means than tick bite, these observations suggest that blood virus will not easily infect through the normal unabraded skin, but, on the other hand, indicate that tick virus can do so readily. We believe that this penetrating power of tick virus has been responsible for part at least of four laboratory cases, apparently without tick bites, that have come under our personal observation. Of five similar cases with which we have had no personal contact, three are known to have been working with infected ticks; two were not. Of the latter there was in one case strong presumptive evidence of the contamination of an abrasion with blood virus, but of the other case there was no information. The danger of becoming infected through an open skin lesion has always been recognized.

Cases contracted in nature without history of tick bite are reported not infrequently and it is beyond question that a bite has nevertheless occurred in the great majority of these cases. There have been some, however, in which the negative report has possibly been correct. In connection with such cases, the contamination of the skin with tick virus, either by the actual mashing of infectious ticks, either on one's person or on an animal host, and the transfer of such infectious material to some easily penetrated portion of the skin, or to the conjunctiva, is a possibility which hereafter should be considered. Such contamination might easily occur during the handpicking of ticks from domestic stock, a practice which is common throughout the tick-infested region.

In view of the results of these tests, it seems unnecessary to assume the occurrence of any other natural vector than *D. andersoni* in order to explain certain human cases.

IMPROVED METHOD OF MANUFACTURE OF THE VACCINE AND A STUDY OF ITS PROPERTIES

By R. R. Spencer, Surgeon, and R. R. Parker, Special Expert, United States Public Health Service

The technic involved in the preparation of the preventive vaccine from fed-infected ticks was at first necessarily crude. The major part of our vaccine supply is still being prepared from adult male and female ticks which have been reared from the eggs of engorged females. The rearing and infecting of these laboratory-bred ticks have been described in a previous paper. (See p. 28.) After the infected ticks have reached the adult stage the treatment of them is thenceforth the same as that given the wild adults described below in our improved method, with the single exception that the wild adults are infected as adults. This later method has been developed during the past two years, and has gone far enough to prove its practicability. It is less laborious and entails far less danger to the laboratory workers, but the older method can not be given up entirely until a sufficient supply of adults from which to manufacture the vaccine can be obtained each season from nature, and the best conditions determined under which to keep them alive and capable of a second feeding late in the fall or midwinter.

TECHNIC OF VACCINE MANUFACTURE FROM INFECTED ADULT TICKS

Wild adult ticks, collected in the field, are permitted to feed on infected guinea pigs after the onset of the fever, placing about 75 ticks in a wire gauze capsule fastened to each animal. After two days of feeding the ticks are removed and placed at room tem-

perature over moist sand, where they may be kept for several months. Before being used for making vaccine these ticks are again fed, this time on normal animals, for five to six days. This second feeding produces a tremendous increase in the number of minimal infectious doses of the virus per tick. For routine purposes it is not necessary to determine this dosage by the graded injections of the live tick-virus suspensions, as was formerly done.

The partly engorged ticks (male and female) are now ground in a mechanically operated porcelain mortar and pestle with fine quartz sand and a small quantity of physiological salt solution which contains 1.6 per cent phenol and 0.4 per cent formalin. After thorough grinding the whole mass is transferred to a large stock bottle, and an additional amount of the salt solution and preservative is added until the concentration reaches, but does not exceed, four ticks per cubic centimeter. After standing for 48 hours, during which time the preservatives will precipitate most of the protein, an equal volume of physiological salt solution is added. This dilutes the preservatives to 0.8 per cent phenol and 0.2 per cent formalin. At this stage the material is kept for seven days at room temperature. This has been found a sufficient period to kill most extraneous organisms, including sporebearers.

The suspension is then diluted once more by again adding an equal volume of salt solution. This final dilution will contain 0.4 per cent phenol, 0.1 per cent formalin, and the killed virus equivalent of one tick per cubic centimeter. The sand, chitin, and precipitated protein is now removed by centrifugation and the remaining clear amber-colored supernatant fluid is ready for the final containers. Occasionally some precipitate forms afterwards. This can be disregarded, for it does no harm when injected, and besides has protective value. In fact, the discarded heavy precipitates from potent lots may be combined and resuspended in sterile salt solution, again cleared by centrifugation, and the clear supernatant fluid still found to possess high protective value. A number of such lots have been so made and used in human vaccination.

The vaccine is finally tested for potency by inoculating six guinea pigs subcutaneously with 1 c. c. each. After 12 days the animals are given intraperitoneally, a test dose of 1 c. c. of guinea pig blood virus. If four of the six animals do not show symptoms of spotted fever, the vaccine is considered suitable for human use. Sterility tests are made in accordance with the hygienic laboratory procedure for biological products.

It is recognized, of course, that an arbitrary standard of potency of this kind involves several variables and the potency of any two

batches of vaccine is only approximately the same. On account of the great demand for the vaccine, we have occasionally been compelled to use batches which fell below this standard.

Although most commercial bacterial vaccines are prepared by heating the organisms to 56° C., the protective quality of spotted fever virus is entirely destroyed at this temperature. This test has been repeated several times by dividing a potent tick virus emulsion into two equal parts, and treating one with phenol and heating the other to 56° C. for one-half hour. The heated material has always failed to protect.

Preservative.—Many of our first batches of vaccine which were prepared with phenol alone had to be discarded because of contamination with extraneous organisms. It was found later that a potent vaccine could also be prepared by using formalin or alcohol as a preservative, but these agents failed to give a clear final product. We then resorted to the use of a double preservative (phenol and formalin), and the occurrence of contaminated lots since has been extremely rare. The final product is now clearer than formerly, is sterile, and there is no measurable reduction in potency. The presence of formalin (0.1 per cent) produces a slight stinging for about five minutes after injection, but otherwise there is no apparent objection to its use.

Effect of centrifugation of the vaccine upon its potency.—Many batches of vaccine have been free of visible suspended matter (turbidity less than 50 parts per million). Furthermore, microscopic examination of the centrifuged sediment reveals no rickettsiæ of any kind, and when such vaccine is centrifuged at 3,000 revolutions per minute, for one hour, and the top portion carefully removed, there is no appreciable difference between the protective value of the top and bottom portions. This is indicated by the following tests:

A. About 15 c. c. of Vaccine No. 250 was centrifuged at top speed (3,100 revolutions per minute) for one hour. Four cubic centimeters of the top were carefully removed and 1 c. c. injected subcutaneously into each of four guinea pigs; four others each received 1 c. c. from the bottom of the tube. After 12 days all were given 1 c. c. of the same blood virus intraperitoneally with the following results:

Pigs receiving top portion:

1. Immune.
2. Immune.
3. Immune.
4. Immune.

Pigs receiving bottom portion:

5. Moderate fever, recovered.
6. Immune.
7. Moderate fever for 3 days; recovered.
8. Immune.

B. The same procedure was repeated on Vaccine No. 250, using 12 guinea pigs.

Pigs receiving top portion:	*Pigs receiving bottom portion:*
9. Spotted fever; recovered.	15. Immune.
10. Immune.	16. Immune.
11. Immune.	17. Spotted fever; recovered.
12. Immune.	18. Spotted fever; recovered.
13. Immune.	19. Immune.
14. Immune.	20. Immune.

C. A similar test was carried out on Vaccine No. 309, using 20 guinea pigs.

Pigs receiving top portion:	*Pigs receiving bottom portion:*
21. Spotted fever; recovered.	31. Immune.
22. One day fever, immune.	32. Immune.
23. Spotted fever; died.	33. One day of fever; immune.
24. Immune.	34. Mild fever; recovered.
25. Mild fever; recovered.	35. Mild fever; recovered.
26. Spotted fever; recovered.	36. Spotted fever; recovered.
27. Immune.	37. Immune.
28. Immune.	38. Immune.
29. Immune.	39. Immune.
30. Mild fever; recovered.	40. Two days of fever; recovered.

Tests A and B indicate that the top portion possessed a greater protective value than the bottom portion. In test C, however, there is very little difference in the protection conferred. We have previously shown that guinea pigs revealed a great variation in the manner of reacting to the vaccine, and the differences in protective value between top and bottom portions can easily be accounted for by the normal variation in susceptibility and the reaction of individual animals. However, from the results of the above test it seems that there is slight danger of losing the protective value of the vaccine in the process of removing the sediment by centrifugation, despite the fact that the sediment, which consists largely of infected tick tissue, also has protective value.

Relation of antigenic power of the vaccine to concentration of the live virus and to the presence of rickettsiae in ticks from which the vaccine is prepared.—Our early studies demonstrated that the live virus in individual hibernating ticks was rarely infectious, but very frequently immunized the animals into which the suspended viscera of such ticks were inoculated. Suspensions of large numbers of hibernating, infected ticks, also fail to give a frank infection, but the animals are nearly always subsequently immune. (See p. 5). Our Experimental Virus Test No. 245 illustrates this.

Two guinea pigs were inoculated intraperitoneally on January 21, 1927, each with the viscera of 10 infected ticks. The ticks had been placed outdoors in glass cylinders in March of the previous year

and had so remained throughout the summer and until tested in midwinter. The temperature of one animal reached 40° C. on the eighth day and then gradually fell. The other had a mild fever for three days which then subsided. Neither showed external evidence of the disease but both were immune to subsequent injections of blood virus. Here it is plain that the virus from 10 infected ticks was not sufficient to give a typical infection but possessed the power to immunize. Control tests on ticks from this same lot showed that, after feeding, the live virus increased to 10,000 minimal infectious doses per tick. The virus in hibernating ticks is, therefore, seen to be passing through a nonvirulent immunizing phase which is, nevertheless, potentially highly virulent.

No close relationship has been found between the amount of live virus in such ticks, as determined by injection of graded dilutions into animals, and the antigenic or protective power of the vaccine prepared from the same virus suspension by the addition of phenol. Many tests, two of which will be given, have demonstrated this.

Seventy infected adult ticks were permitted to feed five days and then were ground in a mortar to a fine suspension. Vaccine was prepared from this suspension in the usual way, first removing a sample for titration of the live virus content. A series of guinea pigs received dilutions (two pigs to each dilution) of the virus, equivalent to that in 1/10, 1/100, 1/500, 1/1,000, 1/5,000, 1/10,000, 1/15,000 part of a tick. All animals up to and including those receiving 1/10,000 dilution developed spotted fever, demonstrating a rather high concentration of virus. Vaccine prepared from the same suspension was given to six guinea pigs, each receiving 1 c. c. or 10,000 killed doses. All of these animals died of spotted fever when given 1 c. c. of blood virus 10 days later. Therefore the vaccine possessed no protective quality, although prepared from a tick virus suspension containing 10,000 doses per cubic centimeter.

The next test shows that vaccine of good antigenic quality may be prepared from tick suspensions containing relatively small amounts of live virus, and, further, that the number or rickettsiæ that can be demonstrated in such ticks is not necessarily related to the degree of protection.

Eleven infected ticks were fed three days and their ground viscera suspended in salt solution as before. A titration of the virus content gave infection to guinea pigs receiving the 1/10 dilution, but none of the animals receiving greater dilutions developed fever. The six animals that each received 1 c. c. of vaccine prepared from the same suspension were all immune subsequently to blood virus. Of course, the amount of blood virus given in the immunity test is not always comparable in any two experiments, but repeated experience clearly

indicates the validity of the conclusion that may be drawn from this test; namely, that the amount of protective antigens in vaccine is not always comparable to the amount of infectious live virus originally in the same material.

Again, each of the 11 ticks from which the virus and vaccine were prepared were first carefully dissected and smears made of six organs; namely, the salivary glands, intestines Malpighian tubules, muscles, brain, and reproductive organs. In five of these ticks no rickettsiæ could be found and only a few in the others—chiefly in the muscles, Malpighian tubules, or intestines. An abundance of rickettsiæ, which is so often encountered in fed-infected ticks, does not seem to be essential to the preparation of a vaccine of good quality, since all six guinea pigs were protected by the vaccine prepared from these ticks.

Is the phenolized virus killed or merely attenuated?—Inasmuch as previous investigators of Rocky Mountain spotted fever, as well as those studying typhus fever, poliomyelitis, rabies, and other diseases of obscure etiology, have suggested that a satisfactory immunity can be conferred only when the antigen contains live virus, the question arises here as to whether or not the virus in our phenolized tick suspensions is dead or merely attenuated.

The following observation, we believe, throws some light on the question:

Unapparent infection may be demonstrated in Rocky Mountain spotted fever as in typhus fever by the injection of an immune serum-virus mixture. Animals so inoculated are apparently unaffected, but the existence of infection may be proven by killing them and injecting suspensions of the ground spleens into fresh animals which promptly develop a typical spotted fever.

Therefore, six guinea pigs were inoculated intraperitoneally with an immune serum-virus mixture. Six others were given 1 c. c. of vaccine No. 290, which had been proven to be highly potent. None of these 12 animals showed evidence of infection. After seven days they were all 12 killed, and a spleen emulsion from each injected into a normal guinea pig. The spleen emulsions from four of the six guinea pigs that had received the serum-virus mixtures produced spotted fever and the emulsion from one other produced an immunity. On the contrary, none of the six guinea pigs receiving the emulsions of spleen from the animals that had been inoculated with the vaccine developed spotted fever.

This test suggests that the phenolized vaccine is free from active virus. It would seem very remarkable if the virus were found to survive in the tick suspensions which contain at first a concentration of 1.6 per cent phenol and 0.4 per cent formalin; and in view of this

result we are inclined to believe that the killed as well as the live virus of Rocky Mountain spotted fever can immunize.

Modification of the fever by vaccination at the time the animals are infected.—So far as our tests have gone, the vaccine possesses no therapeutic value, and best results are obtained when the vaccine is given at least 10 days prior to the onset of the infection. However, Figure 9 shows that the severity of the infection can be greatly modified by vaccinating guinea pigs immediately after the virus is given. Temperatures above 39.6° C. are considered as fever.

By comparing the height and duration of the fever in the first four control animals with the fever in the eight vaccinated animals it may

FIGURE 9.—Vaccination of guinea pigs immediately following infection with virus

be seen that the latter had very much milder infections. Furthermore, it was observed that the vaccinated animals were livelier than the not-vaccinated controls and showed no external lesions, while all the controls developed swelling and discoloration of the scrotum.

Various methods of vaccination.—Vaccination by the subcutaneous, intracutaneous, intramuscular, intraperitoneal, and intravenous routes has given equally good protection. A moderate degree of immunity has been conferred by rubbing the vaccine into the shaved skin for three successive days. Attempts to vaccinate animals by forcing them to swallow the vaccine has only produced immunity in a few instances, and failed in the great majority of cases.

Keeping quality of the vaccine.—The vaccine seems to be fairly stable at ice-box and room temperatures. Satisfactory tests, how-

ever, upon the duration of the potency under various conditions have not yet been carried out, chiefly because of pressure of more important work and because we have been compelled to use our best lots, suitable for such a test, to supply the great demand for human vaccination.

We have tabulated below some of the tests that have been completed so far. The table suggests that some batches of vaccine will retain full potency for more than a year when kept in the ice box. At room temperature potency diminishes more rapidly as a rule, but on the whole the vaccine is a reasonably stable product. All vaccinated animals were tested for immunity by giving 1 c. c. of guinea-pig blood virus intraperitoneally, but the actual amount and virulence of the test dose is, of course, only approximately the same in any two tests.

TABLE 26.—*Duration of potency of vaccine; guinea pigs received 1 c. c. of vaccine subcutaneously*

KEPT AT ICE-BOX TEMPERATURE

Test No.	Vaccine No.	Date of manufacture	First protection test, 3 guinea pigs to each test		Second protection test, 3 guinea pigs to each test	
			Interval in days	Result	Interval in days	Result
1	130	Apr. 2, 1925	198	3, immune	595	3, immume.
2	140	Apr. 20, 1925	170	3, immune	576	2, immune. 1, spotted fever; recovered.
3	221	Mar. 31, 1925	200	3, immune	597	1, immune. 2, spotted fever; recovered.
4	223	Apr. 11, 1925	93	3, immune	189	3, immune.

KEPT AT ROOM TEMPERATURE

Test No.	Vaccine No.	Date of manufacture	First protection test, 6 guinea pigs to each test		Second protection test, 6 guinea pigs to each test	
			Interval in days	Result	Interval in days	Result
5	177	Jan. 21, 1926	6	5, immune 1, valueless	215	1, immune. 4, died of spotted fever. 1, valueless.
6	178	do	6	4, immune 2, mild spotted fever; recovered.	215	4, immune. 1, died of spotted fever. 1, valueless.
7	179	Jan. 22, 1926	5	4, immune 2, milds potted fever; recovered.	108	5, immune. 1, valueless.
8	180	Jan. 23, 1926	6	5, immune 1, died of spotted fever	93	3, immune. 1, spotted fever; recovered. 1, valueless.
9	186	Feb. 1, 1926	7	5, immune 1, valueless	57	4, immune. 1, mild spotted fever; recovered. 1, valueless.
10	192	Feb. 9, 1926	18	6, immune	56	6, immune.

Duration and degree of immunity.—While we have shown that the degree and duration of immunity conferred by the use of the same amount and same lot of vaccine varies with the individual animal, on the whole, we believe a greater degree of protection is afforded within limits by multiple doses than by a single dose. From the work of previous investigators, this is what one would expect. For example, Harvey and Iyengar,[20] from studies upon chicken cholera, have concluded that "single doses of vaccine can produce immunity, but of not so high degree as two or three doses," and that "reimmunization may be carried out with doses which are smaller than even the smallest dose originally required to confer it and is as great and lasts as long as the original immunity." We have not been able to successfully complete similar tests upon the degree and duration of immunity in Rocky Mountain spotted fever, because the virus dosage can not be accurately measured as in a culture. However, our human vaccinations, carried on during the past three years, suggest that the degree of immunity increases proportionately with the number of injections received. While only 15 cases have occurred among a vaccinated group of over 4,600 people, spotted-fever infection has been clinically milder among those who have received multiple vaccinations over a considerable period, in comparison with the symptoms in those who had received only one or two injections.

In view of these results, the vaccine will in the future be manufactured in a more concentrated form and in the approaching season (1929), it will be recommended that the dosage be increased, and that as heretofore vaccination be repeated each year.

The following directions accompany each lot of vaccine sent to physicians:

Preservation.—The vaccine should preferably be kept in a cool place but not allowed to freeze. However, it will remain potent several months at room temperature. It should not be used after nine months from date of manufacture.

Administration.—Withdraw the vaccine by inserting a sterile hypodermic needle through center of stopper. Do not remove rubber stoppers from bottles. Disregard any small amount of sediment that has formed. This sediment is harmless and will disappear when bottle is thoroughly shaken.

Dosage, adults.—The vaccine should be administered subcutaneously in two doses of 2 c. c. each at 5-day intervals. Longer intervals between doses does no harm. More than two injections may be safely given and is indicated in areas of high mortality.

Dosage, children.—Children over 10 years may be given the full adult dose. Children under 10 should be given 1 c. c. at each dose. It is not necessary to vaccinate babies under 1½ years old unless exposed to unusual danger from tick bites.

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[20] Harvey and Iyengar. Development, Duration, and Restoration of Immunity, 1928. Indian Jour. Med. Res., Vol. XV, No. 4, pp. 935–949.

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Vaccine of no therapeutic value.—The vaccine is of no value in treatment and should be given at least 10 days before exposure, but it may modify the course of infection if given several days before the onset of symptoms, especially in areas where the mortality rate is low.

Duration of protection.—Protection may not last longer than one season. Experimental evidence indicates that both the duration and the degree of protection varies widely. It is therefore recommended that the vaccine be taken each year before the beginning of the tick season. Each successive year's vaccination seems to increase the degree of immunity.

Reaction from vaccine.—As a rule, the vaccine produces only local symptoms of redness, swelling, and itching. Headache, slight fever, and general malaise for 24 to 48 hours are occasionally noted. In rare cases an urticarial rash with intense itching may follow. Such cases have cleared up without serious consequences.

Distribution of vaccine.—The vaccine is distributed by the United States Public Health Service to physicians only. Since the material is provided free, it is not expected that any charge will be made for it.

Records.—Please fill out and return the vaccination cards which are sent with the vaccine and report to your health officer any cases of spotted fever occurring among vaccinated persons.

Those who have had spotted fever need not be vaccinated.

RESULTS OF FOUR YEARS' HUMAN VACCINATION

By R. R. Parker, Special Expert, and R. R. Spencer, Surgeon, United States Public Health Service

The preparation of a protective vaccine against Rocky Mountain spotted fever from the tissue virus of infected wood ticks (*Dermacentor andersoni* Stiles), its successful use in guinea pigs, rabbits, and monkeys and its harmlessness in human administration have previously been reported (pp. 28–36).

During the seasons of 1925, 1926, 1927, and 1928, this vaccine has been used experimentally for human prophylaxis. Since the severity of infection varies markedly in different sections of the Rocky Mountain region in which it is endemic, test areas were selected that would afford opportunity to observe prophylactic value against the two extremes of virulence. One was a section of the Snake River Valley of southern Idaho in which the death rate is about 5 per cent; the other, the west side of the Bitter Root Valley, of western Montana, in which the mortality is seldom less than 80 per cent.

The determining factor in the selection of the Snake River Valley area was that local conditions were such that if the vaccine were of full protective value against the mildest types of infection, this fact could be demonstrated more easily and more quickly than in any other section where the disease is endemic. This was possible because the average annual incidence was higher than in any other endemic area of equal size. Of further advantage was the fact that so large a percentage of the cases occurred among men handling sheep that

it would be possible to limit the test to persons engaged in the sheep industry so that only a very small proportion of the resident population need be vaccinated. The plan for the test was accordingly simple; a part of the nonimmune sheep herders and camp tenders that handled sheep on the range, where exposure occurred, were to be vaccinated; those not vaccinated were to serve as controls. Full or probable full protective value respectively would be indicated if none or few cases occurred in the vaccinated group and case incidence among the controls was considerable. If, on the other hand, the vaccine should afford only partial protection, the results would be less instructive, since the natural prevalence of mild infections in this area would tend to minimize the significance of any apparent amelioration of symptoms in vaccinated cases except as it was observed over a period of several years.

In the Bitter Root Valley area both conditions and procedure were different. The average annual incidence was low (5.75 cases per annum, 1917 to 1928), the disease was of almost constant high virulence with few recoveries, and no important proportion of cases was limited to any one industry. Provided protection were only partial, the nearly uniform severity of infection with high death rate made this area a peculiarly favorable one in which to test for amelioration of symptoms. Low incidence and the lack of restriction to any particular occupation, however, demanded that as high a proportion as possible of the resident exposed population be vaccinated. However, because of the low incidence, complete protection in this area would be difficult to show, even though a large number of persons were vaccinated. The results of spotted fever control operations—cattle dipping, rodent control, etc.—which have been carried on for a number of years by the State would also tend to make difficult the interpretation of decreased case incidence. On the other hand, if there should be partial protection the recovery of even a relatively small number of vaccinated individuals would be of definite significance, provided the usual high death rate were maintained at the same time among the nonvaccinated.

The surplus of vaccine not needed in the test areas has been distributed upon request to physicians in other parts of the Northwest.

A total of 3,893 persons have been vaccinated.

The dosage used has necessarily been purely arbitrary and not at all constant. For initial immunization of adults, two injections of 2 c. c. each, five days apart, have most frequently been used. This is equivalent to the phenolized virus content of four adult ticks in which the virus has been "reactivated" (see p. 7) and brought to its maximum infectiousness by several days of tick feeding, the average virus content per tick being 5,000 to 15,000 minimum in-

fectious doses for a guinea pig. For subsequent vaccinations, the same dosage was repeated in the Bitter Root Valley but only one injection was given in southern Idaho.

The results of these two tests and of the use of the vaccine in other sections have been distinctly encouraging. Against the highly fatal strains, such as occur in the Bitter Root Valley, they show a partial protection sufficient to alter markedly the clinical course of infection and in most cases to insure recovery. Against the relatively very mild strains, such as those prevalent in southern Idaho, they strongly suggest that full protection is usually conferred.

The duration of protection in most persons, as indicated by the power of the blood to neutralize the virus, is usually short and therefore vaccination each year is desirable, especially in areas in which infection is of the virulent type. There is evidence, however, that in some persons, at least, a greater or less degree of residual immunity persists from one season to the next.

Bitter Root Valley Test

In the Bitter Root Valley area 34 [21] persons were vaccinated in 1925, 654 in 1926, 1,296 in 1927, and 812 in 1928. Of these figures 745 represent duplications, 1,469 persons having been vaccinated once, while 430 have been vaccinated in two seasons, 141 in three, and 11 in four. The total of vaccinated individuals, then, has been 2,051. These were separable into two groups on the basis of exposure to danger of infection.

GROUP A

A group of not more than 75 laboratory and field workers have been engaged in Rocky Mountain spotted fever studies since investigations of the disease first began about 1902. Prior to the use of the vaccine (before 1924), three cases occurred among employees of the United States Public Health Service, one case in an employee of the Montana State Board of Health and one case at the Rockefeller Institute in New York City. All five were fatal. In 1927, another fatal case occurred in a laboratory worker (also not vaccinated) at the University of Berlin.

Our vaccinated Group A, 59 in number, consisted of the research personnel of the United States Public Health Service spotted-fever laboratory and the staff of the Montana State Board of Entomology, these organizations occupying joint headquarters at Hamilton in the Bitter Root Valley. These persons were exposed to an unusual danger of infection due to the nature of their work, especially certain

[21] Previously reported but the significant data is included in the present paper for the sake of completeness.

of the Public Health Service personnel who were engaged in rearing hundreds of thousands of infected ticks for vaccine production. This group comprises, therefore, over three-fourths of all persons who have ever been engaged in Rocky Mountain spotted-fever investigations. Seven cases occurred among this group with one death, during the 4-year period. None of the six cases that recovered was at any time in serious danger and as compared with the usual local case in nonvaccinated individuals, there was less evidence of toxemia, the heart and kidneys were less severely affected, the eruption did not become hemorrhagic, mental depression was absent or slight, the usual anxious expression was absent or much less apparent, and nervous symptoms and insomnia were less in evidence. There is no reason to believe that our laboratory strains with which these cases were infected had become less virulent than the strains with which the previous fatal cases were infected. Indeed, the virulence of all strains to laboratory animals was kept up and weak strains always discarded in order to insure a potent virus for use in making vaccine. All the nonvaccinated laboratory cases died in eight days or less, and if the death rate among laboratory workers is a safe guide, we must consider laboratory strains at least as virulent as those encountered in nature.

No definite explanation for the failure of the vaccine in the one fatal case (V) can be offered. There are, however, several possibilities: First, that the patient did not respond to the injected antigen; second, that some degree of immunity was conferred, but was of short duration; and, third, that the vaccine used in September, 1927, and which had been prepared in April, had lost its potency in whole or in part. Experience indicates slight, if any benefit could have resulted from the injection of January 30, which must have been given either shortly before or shortly after infection occurred. We give below the clinical histories of the seven cases among vaccinated laboratory employees.

CASE HISTORIES—GROUP A

Case 1, 1926

F. P. M., Hamilton, Mont., age 34, laboratory attendant at Rocky Mountain Spotted Fever Laboratory of the United States Public Health Service at Hamilton.

Vaccination record.—1925: April 23, 1 c. c. of vaccine No. 130, each c. c. of which contained the killed virus content of two ticks. No further injection was given because of allergic reaction.

Exposure.—Patient was engaged in rearing infected ticks for vaccine production. He became infected in January, 1926, nearly nine months after vaccination, but had no knowledge of tick bite or other mode of infection.

Clinical history.—Headache was experienced on January 18; on the 19th there were chilly sensations, tired feeling, and inclination to stretch; muscular pains developd on the 20th, and patient worked only part time. On the 21st symptoms became aggravated and physician was called. Temperature was about 105° F., pulse 110; ankles showed hyperemic spots; conjunctivæ were congested. Diagnosis of Rocky Mountain spotted fever was made and patient taken to hospital. Temperature and pulse rate dropped following hospitalization, and pulse only once exceeded 90 thereafter. On the 22d, the eruption had spread to the forehead, arms, and legs. The spots were quite numerous and of a fair size, but rather faint. They gradually faded. On the 25th the temperature, which had dropped somewhat during the two preceding days, rose to 103° F., but again declined on the 26th, following the appearance of still another crop of spots. Illness was attended by mild delirium; lumbar pain, cough, and constipation were present. The hearing was affected. Urine remained free of albumen. Restlessness was rather pronounced. Temperature first returned to normal on February 4, 17 days after onset, but there were afternoon rises for five days more. He was discharged February 15. (Fig. 10, Case I.)

Convalescence was rapid and patient was able to perform full-time work less than one month after leaving hospital.

CASE II, 1926

A. M. C., Hamilton, Mont., age 62, janitor at the Rocky Mountain Spotted Fever Laboratory of the United States Public Health Service at Hamilton.

Vaccination record.—1926: March 26, 2 c. c., and April 1, 2 c. c., both injections of vaccine No. 173.

Exposure.—Patient was bitten by a tick in the laboratory on August 7, four months and six days after second injection.

Clinical history.—Patient first felt ill on August 13, with weakness, headache, and chilly sensations. Although these symptoms became aggravated he remained at work until the 15th, when he first complained. At that time the conjunctivæ were congested; temperature was 100.4° F., pulse 54 (normal pulse rate 40). Physician was called and patient taken to hospital. On the 17th a characteristic eruption of rather large, scattered spots appeared on lower limbs and back. Additional eruptions, more generally distributed and extending to the hands and fingers, appeared on the 20th and 24th, and in each instance was followed by a drop in temperature. Eruption remained scattered and, except as the successive crops faded, the spots remained bright in color.

During the illness, constipation, tremor, and muscular pains were present. Patient was slightly delirious and hearing was slightly impaired. There was no albuminuria. Coma was absent. Temperature returned to normal August 27, 15 days after onset. Patient was discharged from the hospital September 4, and returned to work November 1. (Fig. 10, Case II.)

A. M. C. was the first person to become infected who had received the full experimental dosage of vaccine, 4 c. c. Infection was contracted a little over four months after vaccination. The clinical course was milder than in the previous case, but not as mild as Cases III, IV, and VI, who had been vaccinated, respectively, two, three, and four successive years, nor as in Case VII, who had received 8

c. c. the same year that he became infected. Four of the five naturally infected cases (IX to XII) were also more mild.

It is significant that this patient was a man of 62 years of age and in poor general health. *This is the first record of recovery of a person of advanced years infected with the Bitter-Root type.* He returned to work 57 days after leaving the hospital. Recovery was somewhat slow due both to his age and to two setbacks that resulted from overexertion.

CASE III, 1926

M. L. N., Hamilton, Mont., age 24, laboratory attendant at the United States Public Health Service Rocky Mountain Spotted Fever Laboratory at Hamilton.

Vaccination record.—1925: April 26, 1 c. c. of vaccine No. 130; May 3, 1 c. c. of vaccine No. 223. 1926: March 31, 2 c. c. of vaccine No. 180; April 5, 2 c. c. of vaccine No. 195.

Exposure.—Like Case I, this patient was engaged in rearing infected ticks for vaccine production. He was tick bitten the night of August 23.

Clinical history.—The onset was sudden. Patient became ill during the afternoon of August 27, with severe frontal headache and increasing malaise. During the evening there were alternate periods of sweating and chilly sensations; back of neck was sore and patient complained of pains in joints of right arm. In the morning he was no better. Physician was called, and he was taken to hospital. Temperature reached peak of 103.4° F. on the night of 29th, then declined with general amelioration of symptoms reaching normal the morning of September 1. Thus far the only evidence of eruption was about 20 medium sized hyperemic spots on the legs above the ankles. From early morning of September 1 to the afternoon of the 4th, temperature at no time exceeded 99° F. On the latter date patient was up and about the hospital expecting to go home. In the afternoon, however, temperature began to rise and a typical eruption appeared. The spots were few in number, well scattered and mostly confined to extremities. Temperature reached peak of 103° F. on the 7th, then declined reaching normal on the 13th, 17 days after onset. (Fig. 10, Case III.)

During the second febrile period the symptoms were much the same as during the first except that they were slightly more aggravated and that in addition patient complained of generalized muscular soreness; pains in joints were absent. There was a slight soreness of eyeballs to touch. There was no albuminuria, and hearing was not impaired.

Patient was discharged September 19, and returned to do full-time work October 6, 17 days after leaving the hospital.

During illness patient was remarkably free from the usual distressing symptoms. The conjunctivæ were not congested, and he was able to read and smoke each day. There was no delirium, and nervous symptoms were slight. There was but one night that patient did not secure good rest.

Blood drawn September 8 failed to infect guinea pigs.[22] It is of interest to note that on this date, following the use of a tourniquet applied above the left elbow, there was cyanosis of the forearm and petechial spots appeared, the forearm presenting an appearance typical of that frequently seen in severe cases.

Case III was the first to occur in a person vaccinated in more than one year. The course of infection was milder than in Cases I and II and, of the later *laboratory-infected* cases, has been exceeded in mildness only by Case VI, who was vaccinated in four successive years. Loss of weight was slight and recovery was rapid.

Compare clinical record of Case VII, who had a similar 2-febrile-period course of infection.

CASE IV 1927

L. H. McN., Hamilton, Mont., age 30, laboratory attendant, United States Public Health Service Rocky Mountain Spotted Fever Laboratory at Hamilton.

Vaccination record.—1925: April 26, 1 c. c. of vaccine No. 130; May 3, 1 c. c. of vaccine No. 223. 1926; April 2, 2 c. c. of vaccine No. 186; April 7, 1 c. c. of vaccine No. 195. 1927; February 10, 2 c. c. of vaccine No. 218AB.

Exposure.—Like Cases I, III, VI, and VII, this patient was engaged in rearing infected ticks for vaccine production. On April 3, he removed from the scrotum a tick which he thought might have been attached two days. He failed to report this fact and destroyed the tick.

Clinical history.—Patient became ill April 13, following an incubation period of 10 days reckoned from the date the tick was removed. He returned to work April 14, but became much exhausted on the slightest exertion. At 2 p. m. his temperature was 101° F. and there was slight headache, muscular pains especially in back of neck and lumbar region, chilly sensations, congested conjunctivæ, and malaise. Spotted fever was suspected and physician called. Patient remained at home till April 16, when he was hospitalized. He complained of occipital headache and stiffness in joints. The single crop of spots appeared April 18, and consisted of relatively large, bright, scattered spots, generally distributed. Temperature reached a peak of 104.2° F. on night of April 16, and first became normal April 28, 15 days after onset. During the course of illness there was no marked toxemia, the patient was slightly delirious on but one occasion and kidney complications were absent. He was first able to sit up on May 1, but did not leave hospital until May 17. He returned to work June 7, after an absence of 55 days. (Fig. 10, Case IV.)

This case, though relatively mild as compared with the usual non vaccinated case, was more severe than would have been expected considering the amount of vaccine given. This may have been due in part to a probable massive infection, since the infecting tick, which is presumed to have been attached at least two days was likely one in which the virus had already been " reactivated " before attachment took place. It is also a fact that the last injection of vaccine, which was given February 10, 62 days before onset, was from a lot which

[22] Blood from mild cases frequently is noninfectious.

did not measure up to the minimum standard of potency. This patient's return to normal health was slower than that of any of the prior cases except Case I; much slower in fact than the apparent severity of infection would lead one to expect, and was characterized by nervousness, absence of appetite, and lack of endurance.

Blood taken on April 16, on the fourth day of fever, failed to infect guinea pigs, but a sample secured May 5, 23 days after onset, when mixed with active virus fully protected three guinea pigs into which the mixture was injected.

CASE V, 1928

A. L. K., Hamilton, Mont., age 22, bacteriologist at the United States Public Health Service, Rocky Mountain Spotted Fever Laboratory at Hamilton.

Vaccination record.—1927: September 1, 1 c. c. of vaccine No. 290; September 6, 2 c. c. of vaccine No. 290. *1928:* January 30, 1 c. c. of vaccine No. 304.

Exposure.—This patient for the most part made routine laboratory tests, did not handle infected ticks, and only occasionally worked with infected animals. We have no evidence of the source of infection or portal of entry.

Clinical history.—On retiring on night of February 3, patient complained to roommate that he felt "all in." Was at work on morning of February 4, but looked and felt quite ill. Went to bed in afternoon. On next day there were muscular pains, severe frontal headache, marked prostration, and congested conjunctivæ. Temperature was 103° F. Influenza, which was prevalent at that time, was suspected. During the next two days, there was some improvement, and on morning of February 8, temperature was 99° F., and patient felt markedly better. He was up for a while but felt so completely exhausted that he soon returned to bed. In the early afternoon, temperature within an hour rose from 99° F. to 104° F., and collapse occurred. He was immediately taken to the hospital. A faint eruption was noticeable, and diagnosis of spotted fever was made. On 9th, eruption was typical. Patient from this time on gradually became worse. The eruption soon assumed a purplish color, and by the 11th had become confluent and of typical turkey-egg appearance. The pulse rate gradually mounted, and last three days of illness was between 138 and 160 per minute. Temperature on only one day failed to go above 104° F. On the 13th it reached 106° F., and on 14th 107.6° F. Patient was in coma much of the time and low delirium was frequently present. Death occurred the evening of February 14, 11 days after onset. (Fig. 11, Case V.)

Case V has been the only fatality to occur among 12 vaccinated persons who have become infected during the four years that the vaccine has been used against the virulent Bitter Root Valley strains. During the same period, there have been 15 deaths among 18 non-vaccinated cases.

CASE VI, 1928

W. T. S., Hamilton, Mont., age 52, laboratory attendant at the United States Public Health Service Rocky Mountain Spotted Fever Laboratory at Hamilton.

Vaccination record.—1925: March 29, 2 c. c. of vaccine No. 176; April 3, 2 c. c. of vaccine No. 195. 1926: April 30, 1 c. c. of vaccine ?; May 5, 1 c. c. of vaccine No. 223; May 11, 1.5 c. c. of vaccine No. 223. 1927: March 19,

1.5 c. c. of vaccine No. 253; March 27, 1.5 c. c. of vaccine No. 219AB. 1928: February 13, 1 c. c. of vaccine No. 304; February 18, 1 c. c. of vaccine No. 316; February 23, 1 c c. of vaccine No. 316.

Exposure.—Like Cases I, III, IV, and VII, this patient was engaged in rearing infected ticks for vaccine production. On April 27 he found a tick attached to the abdomen.

Clinical history.—On May 1, four days after finding tick, patient complained of headache and of alternate periods of feeling hot and chilly. Though not feeling well remained at work until the morning of May 6, when he was taken to the hospital as a suspected spotted fever case. At this time he complained of frontal and occipital headache, and aching of bones and muscles; conjunctivæ were congested; eyes were sore to touch and sensitive to light; face was deeply flushed and had an anxious expression so often seen in spotted fever; malaise was considerable. On May 7, symptoms had ameliorated somewhat and improvement continued. The febrile period lasted four days. Highest temperature was 102.6° F., on day of admission to hospital. On May 8 there were a number of suggestive hyperemic spots on the back, but a typical eruption failed to develop. Patient left hospital May 14 and returned to work May 21. During hospitalization the extent of eye involvement was unusual for so mild an infection, and after return to duty patient complained of blurred vision. On August 1 an oculist was consulted who advised that the trouble was due to minute haemorrhagic areas on the retina and that vision would improve as these cleared. (Fig. 10, Case VI.)

Although the typical eruption was not present in this case, we feel that the otherwise characteristic symptoms, the known exposure, the tick bite received in a room where none but infected ticks were being handled, and the concurrence of opinion of the several experienced physicians who saw the patient, fully justified the diagnosis of spotted fever.

Case VI was the mildest of the 12 cases here reported in vaccinated persons contracting the disease from Bitter Root Valley strains.

Case VII, 1928

D. W., Hamilton, Mont., age 25, laboratory attendant at the United States Public Health Service Laboratory at Hamilton.

Vaccination record.—1926: September 3, 2 c. c. of vaccine No. 225. 1928: March 7, 1 c. c. of vaccine No. 315; March 12, 1 c. c. of vaccine No. 316; March 17, 2 c. c. of vaccine No. 314; April 15, 2 c. c. of vaccine No. 330; June 25, 2 c. c. of vaccine No. 353.

Exposure.—Like Cases I, III, IV, and VI, this patient was engaged in rearing infected ticks for vaccine production. On June 24, a fed tick (evidently one that had just completed a virus-reactivating feeding on a guinea pig) was found attached to the flexor surface of right forearm. It was but lightly attached and had apparently been feeding but a short time. Two cubic centimeters of vaccine were given the following day.

Clinical history.—Patient first felt really ill July 3, nine days after tick bite, but had been conscious of lack of vigor since July 1. On afternoon of July 4, went to bed but was up in evening. Worked all day July 5, but felt quite badly in afternoon. On morning of 6th, had severe frontal and occipital headache, face was deeply flushed, conjunctivæ were injected, eyes were sore to touch,

and there was marked prostration. Temperature 102° F. Physician was called and patient immediately taken to hospital. On 7th the above symptoms were accentuated and in addition there was severe muscular aching of neck and back and patient complained of pain in bones and joints. On the 8th, was feeling much better, and subsequent improvement was rapid. Temperature reached 103° F. only once and was normal July 10. There was no evidence of eruption during the above febrile period. He left the hospital July 12 and returned home under physician's orders to remain quiet.

During the next few days there was a painful swelling of right knee accompanied by shifting joint pains, suggestive of acute articular rheumatism. July 18, however, patient felt so well that he walked a half mile to town, and after returning home split wood. Following this heavy exercise temperature rose to 102°F., and on the 19th was 103° F. Patient was again removed to hospital on the 20th. A typical eruption appeared on this date, but though it became generalized was quite scattered and was bright in color as is characteristic of mild infections. Fever was present for eight days but never exceeded 103° F. Constitutional symptoms were more severe than during initial illness. Hospitalization ended July 31.

Patient made rapid recovery and returned to work August 27. (Fig. 10, Case VII.)

This case was of particular interest first, because of the absence of the characteristic eruption during the first febrile period and, second, because of the recurrence, when apparently on the way to recovery, of symptoms in aggravated form with typical eruption, following heavy exercise. The attending physician and the writers were convinced of the specific nature of the infection during the initial illness. The patient, however, was skeptical because of the relative mildness of his symptoms and as stated actively exercised against physician's orders. In our opinion the undue exertion was responsible for the second period of illness, although this can not be positively asserted. In this connection, attention is called to Case III, which ran a similar 2-febrile-period course, the second period immediately following exertion as in Case VII.

GROUP B

During the four years that vaccination has been practiced in the Bitter Root Valley a total of 1,992 persons have been vaccinated outside of group A. The large majority of these vaccinations were done on persons whose exposure to infection was rather uncertain and for whom there was no satisfactory control group. From the total number of vaccinated persons we have therefore selected for study a group of 1,208 persons residing in a well-defined dangerous zone on the west side of the valley. Of these 1,208 persons, 496 were vaccinated, leaving the remainder, 712 in number, to serve as controls. So far as we are able to judge the risk of exposure to infection was identical in these two groups. There were during this

period of four years three cases (VIII, X, and XI) (1 in 165) among the members of the vaccinated group, none of which was fatal, while there were nine cases (1 in 79) among the nonvac-

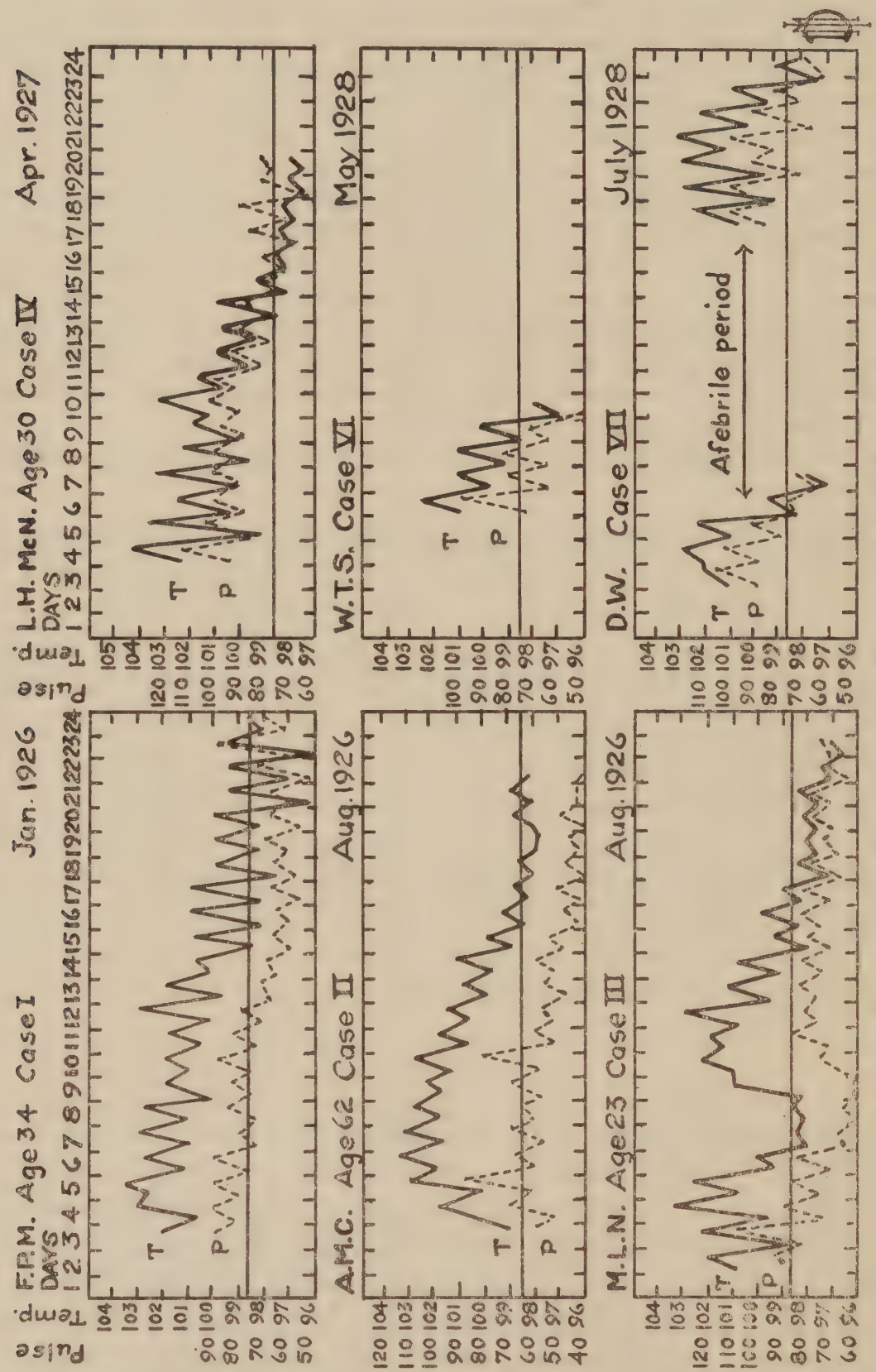

FIGURE 10.—Recovered cases vaccinated—infected with laboratory strain

cinated group, seven of which proved fatal, a case mortality rate of 77.7 per cent.

During the period of the four years under consideration there were a total of 18 cases with 15 deaths, a case mortality rate of 83.3 per cent, among the nonvaccinated persons living in the Bitter Root

Valley. This includes the nine cases with seven deaths in the control group referred to above.

The mortality among the nonvaccinated cases was somewhat higher than the average of the 12-year period 1917 to 1928, the longest for which entirely accurate figures are available. During this period there were 69 cases and 53 deaths, a mortality rate of 76.81 per cent. Fifty-three were adults of whom 45, or 84.91 per cent died; 16 were children, of whom 8 (or 50 per cent) died. In 6 of these 12 years the mortality among adults was 100 per cent.

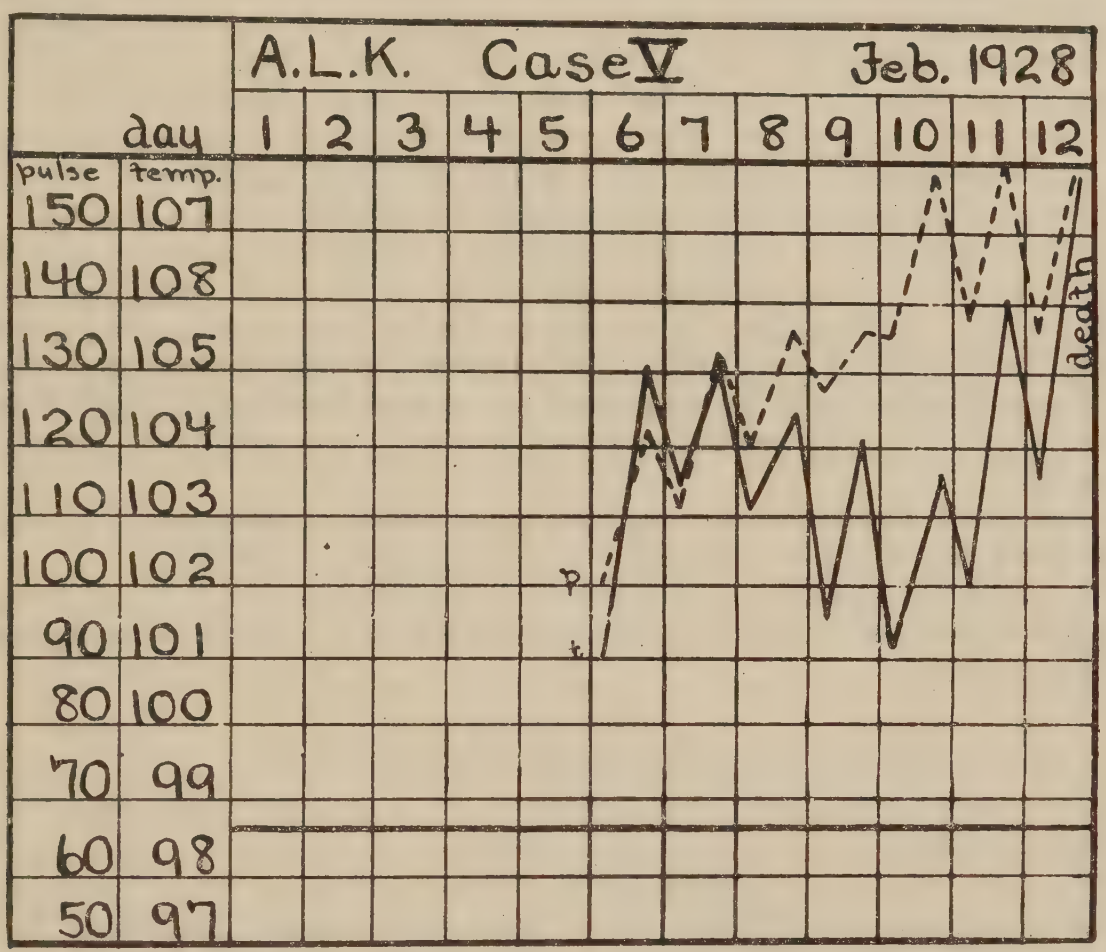

FIGURE 11.—Fatal case—vaccinated—infected by laboratory strain

CASE HISTORIES OF VACCINATED PERSONS IN GROUP B

CASE VIII, 1925

E. O. E., Stevensville, Mont., age 43, district supervisor, State Board of Entomology, Rocky Mountain spotted fever control. This case has previously been reported in detail (see p. 34), and only salient points will be given.

E. O. E. was vaccinated in April, 1925, receiving two injections of 2 c. c. each, respectively, on the 8th and 13th. He became infected from nature (not with a laboratory strain) either the day of or the day before the second injection. Although seriously ill he was never in grave danger, and the clinical course differed markedly from that of the usual highly toxic case characteristic of this locality. The eruption consisted of large, bright spots, well scattered, and were most in evidence on the back. Convalescence was slow. He was unable to perform full-time work for eight months, and did not feel normal for nearly a year.

If the result of the experimental use of the vaccine in laboratory animals is a safe criterion it is unlikely that this man was benefited by the second injection of vaccine, and even doubtful if full benefit was derived from the first. Of the 11 recovered Bitter Root area cases reported, this was the most severe in its effects as indicated by the prolonged period of convalescence.

CASE IX, 1927

J. T. B., Willow Creek, Mont., age 21, field agent United States Department of Agriculture.

Vaccination record.—1927: May 24, 1 c. c., May 29, 2 c. c. vaccine No. 287.

Exposure.—Had been in Bitter Root Valley since middle of June, during which time he twice visited dangerous infected areas—Blodgett Canyon about two weeks and Lake Como about one week before onset. In the latter locality he found ticks on his clothing, but was unaware that he had been bitten.

Clinical history.—Patient became ill July 7, and entered hospital July 10. The entire course was characterized by unusual mildness of symptoms. The eruption consisted of large, scattered, bright spots, not especially numerous and not hemorrhagic. Temperature did not exceed 103° F.; there was no delirium, and patient was mentally alert. His temperature first reached normal July 18, and was normal continuously beginning July 21, 14 days after onset; he left hospital July 29. He was able to return to work August 1, two days later. Chart III, Case IX.

Blood taken July 14, seven days after onset, failed to infect any of the guinea pigs into which it was inoculated.

The distinctive points of this case were its mildness with unimpaired mentality and absence of any marked bodily discomfort, and the rapid return to normal health.

CASE X, 1927

W. McN., Butte, Mont., age 10, nephew of case No. IV.

Vaccination record.—1927: June 20, 1 c. c. of vaccine No. 292; June 27, 1 c. c. vaccine No. 293A.

Exposure.—Since middle of June had been visiting on farm on border of tick-infested area. Had made several trips into Blodgett Canyon, a heavily infected area, within the two weeks period prior to onset. Had no knowledge of being tick bitten.

Clinical history.—Onset August 12, with headache, malaise, and marked muscular pains. Patient was hospitalized August 17. There were two crops of spots, the first appearing August 15, the second August 20, following a rise of temperature to 103° F. Temperature first became normal August 23, 11 days after onset and remained normal after the thirteenth day. Discharged from hospital August 27. (Fig. 12, Case X.)

Patient was feeling normal 5 days after leaving hospital—that is, 24 days after onset—and began school on September 6.

CASE XI, 1928

W. V. T., Darby, Mont., age 37. Employed at a lumber camp.

Vaccination record.—1928: Received 3 c. c. of vaccine No. 331, 1 c. c. on each of the following dates: April 18, 23, and 28.

Exposure.—Was working in a known infected area. On May 22, found two ticks attached to body; one on inner surface of right thigh, the other behind left ear.

Clinical history.—Onset occurred May 25. Patient felt tired out and "ached all over"; and there were alternate periods of chilling and sweating. On the 26th, felt so ill that he went to bed and called physician; temperature was 104° F. Was delirious night of 25th and 26th. On 27th, a characteristic eruption appeared on arms, legs, and back. Complained of being so tender over entire body that he could hardly lie in bed; leg muscles hurt on pressure. Eruption was still visible the next day, but had practically

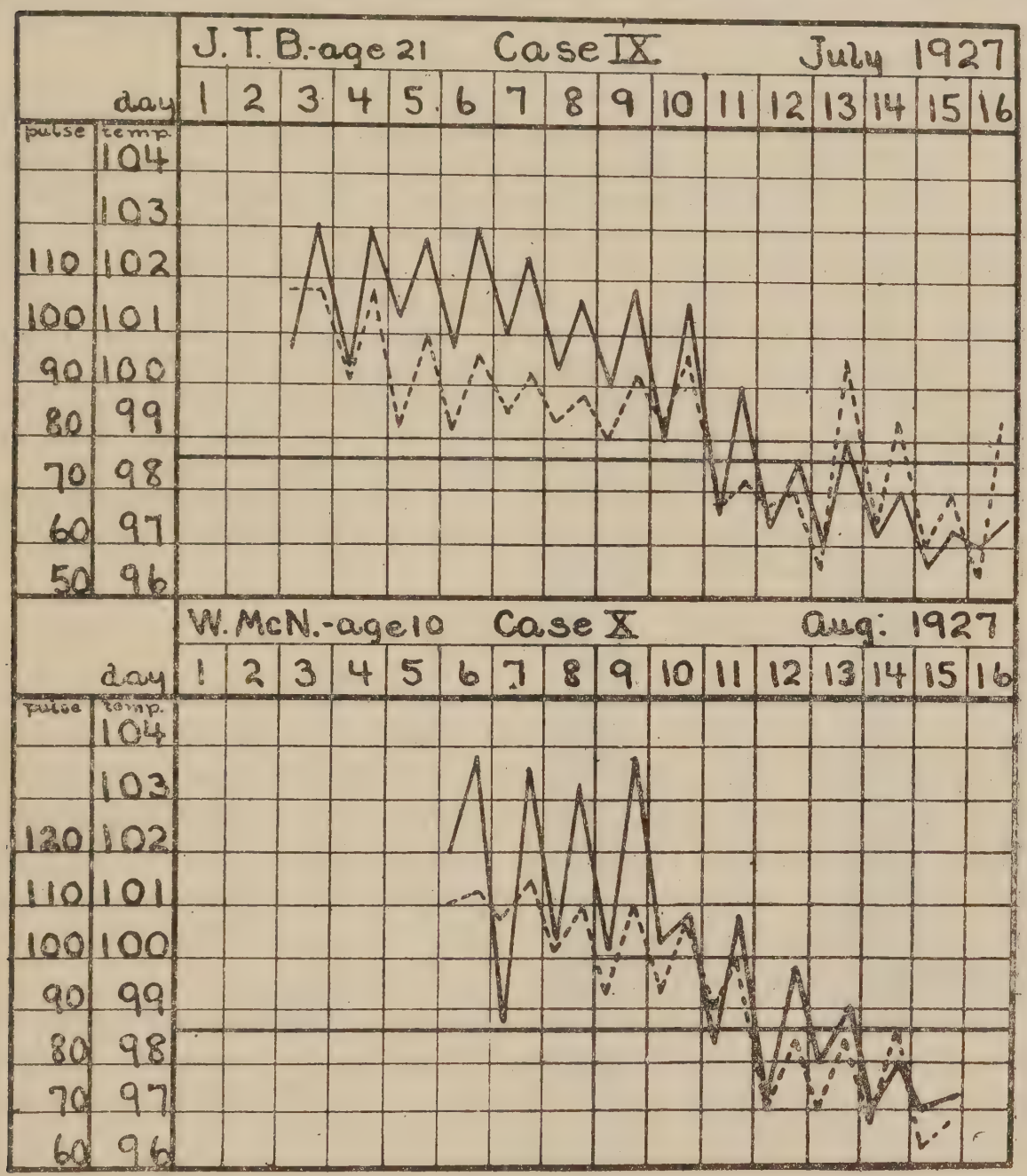

FIGURE 12.—Recovered cases—vaccinated—infected in nature

disappeared on the 29th. Patient felt much better, dressed and walked short distance to post office, but was so weak he had difficulty in getting back to the house. The heart muscle was temporarily quite severely affected, and because of persistent efforts to get up, patient was brought to the Hamilton Hospital June 1. Temperature remained normal during the next three days. He was discharged June 3, and was able to return to work within a few days.

In spite of quite severe symptoms at onset this case was of very short duration, the clinical symptoms persisting for not more than seven days.

CASE XII, 1928

W. H., Hamilton, Mont., age 18. Attending school.

Vaccination record.—1927: Received 5 c. c. of vaccine No. 218AB, 2 c. c. and 3 c. c., respectively, on February 11 and 16. 1928: February 27, 1 c. c. of vaccine No. 314, 1 c. c. and 2 c. c. of vaccine No. 318, respectively, on March 7 and 12.

Exposure.—Patient lived on small farm on west side of Bitter Root Valley, just outside the danger zone that parallels the mountains. When retiring, July 6, found a small tick, which he claims was a nymph, attached in the left axillary region. Thought it probably became attached July 5.

Clinical history.—First felt ill evening of July 7. Was working in hay field and continued in spite of indisposition until July 12, when he went to bed. At that time he was experiencing all the prodromal symptoms of spotted fever. Typical eruption appeared July 14, first on the ankles; was general on the 15th, but remained scattered and bright in color. Mental condition was good throughout illnness and he was more interested in talking of other matters than of his illness. Patient was not hospitalized and hence no detailed record of his illness is available. Temperature reached 104° F. only once. For most part slept well and was restless only a few nights. He first sat up on seventeenth day of illness, and in another week was able to be about. When seen August 25, he stated that he was performing light labor and felt normal except for weakness if he attempted too heavy work.

Though relatively mild as compared with the usual nonvaccinated Bitter Root Valley case, the patient was more ill than any of the four other naturally infected cases, except Case VIII.

DISCUSSION OF BITTER ROOT VALLEY TESTS

Considering only adult cases in the Bitter Root Valley as a whole, the recovery of 10 of 11 vaccinated adults, 7 of Group A and 4 of Group B (90.91 per cent recovery), during the 4-year test period offers vivid contrast, first, with the death of 10 of 11 nonvaccinated adults (90.91 per cent mortality) during the same period, and second, with death of 9 of every 11 nonvaccinated adult cases (84.91 per cent mortality) during the past 12 years. These data are graphically shown in Figure 13.

As compared with the small proportion of naturally infected cases that recover (mostly children), there was a noticeable shortening of the febrile period and, except in Case VIII and to a less degree Case IV, convalescence was more rapid. Even in areas where the so-called mild types of infection prevail, recovered persons seldom regain normal health for a considerable period. As further evidence of the relative mildness of the vaccinated cases is the fact that blood samples taken from 4 of the 12 cases during the febrile period failed to infect guinea pigs, this corresponding to our experience with the mildest types of nonvaccinated cases.

Figure 14, showing the temperature and pulse records of three typical nonvaccinated fatal cases, and Figure 15, showing those of three nonvaccinated recovered cases, are given for comparison with

similar data of vaccinated cases. Figure 10 shows the temperature and pulse records of six recovered, vaccinated, laboratory-infected cases; Figure 12 those for two recovered, vaccinated cases infected in nature; and Figure 11 those for the single fatal vaccinated case. This comparison of charts, however, does not adequately attest the marked amelioration of clinical manifestations in the vaccinated cases, which has been so obvious to the attending physician. The

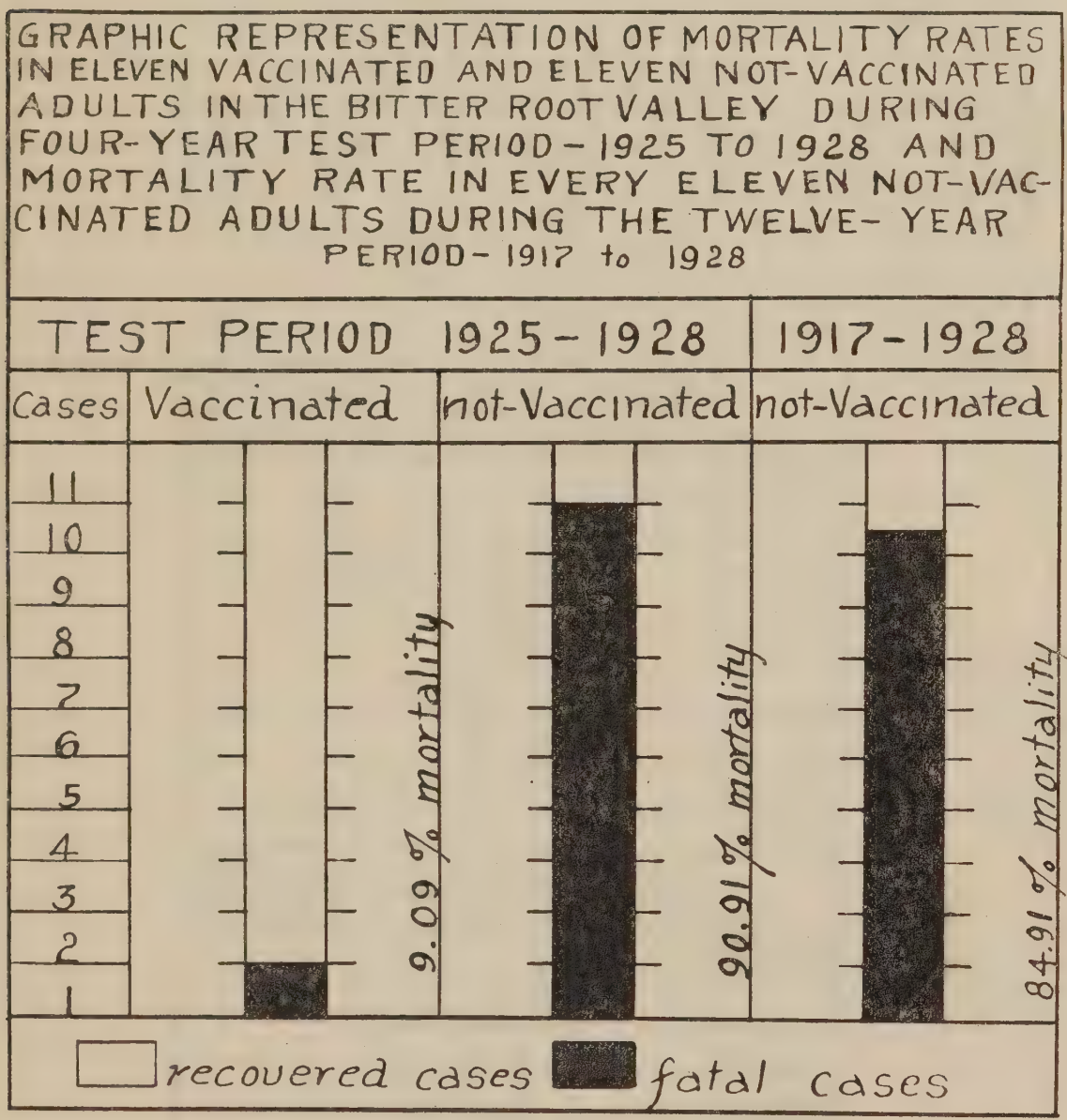

FIGURE 13

usual adult case dies between the seventh and ninth day, although death may ocur as early as 4 days or as late as 20. Generally the prolonged fatal cases are those of children as shown in the case of K. R., age 12, Figure 14. The other records on Figure 14, W. E. G., age 23, and G. H. C., age 38, are of two *fatal, nonvaccinated, laboratory-infected* cases, respectively fatal in seven and eight days, which occurred before the use of the vaccine. It is of especial interest to contrast them with records of the subsequent, *recovered, vaccinated, laboratory-infected* cases of Figure 10.

Comparison of Figures 10 and 12 with Figure 15 show the more protracted periods of illness of recovered, nonvaccinated cases. In the latter group the patients, all children, respectively 16, 8, and 8

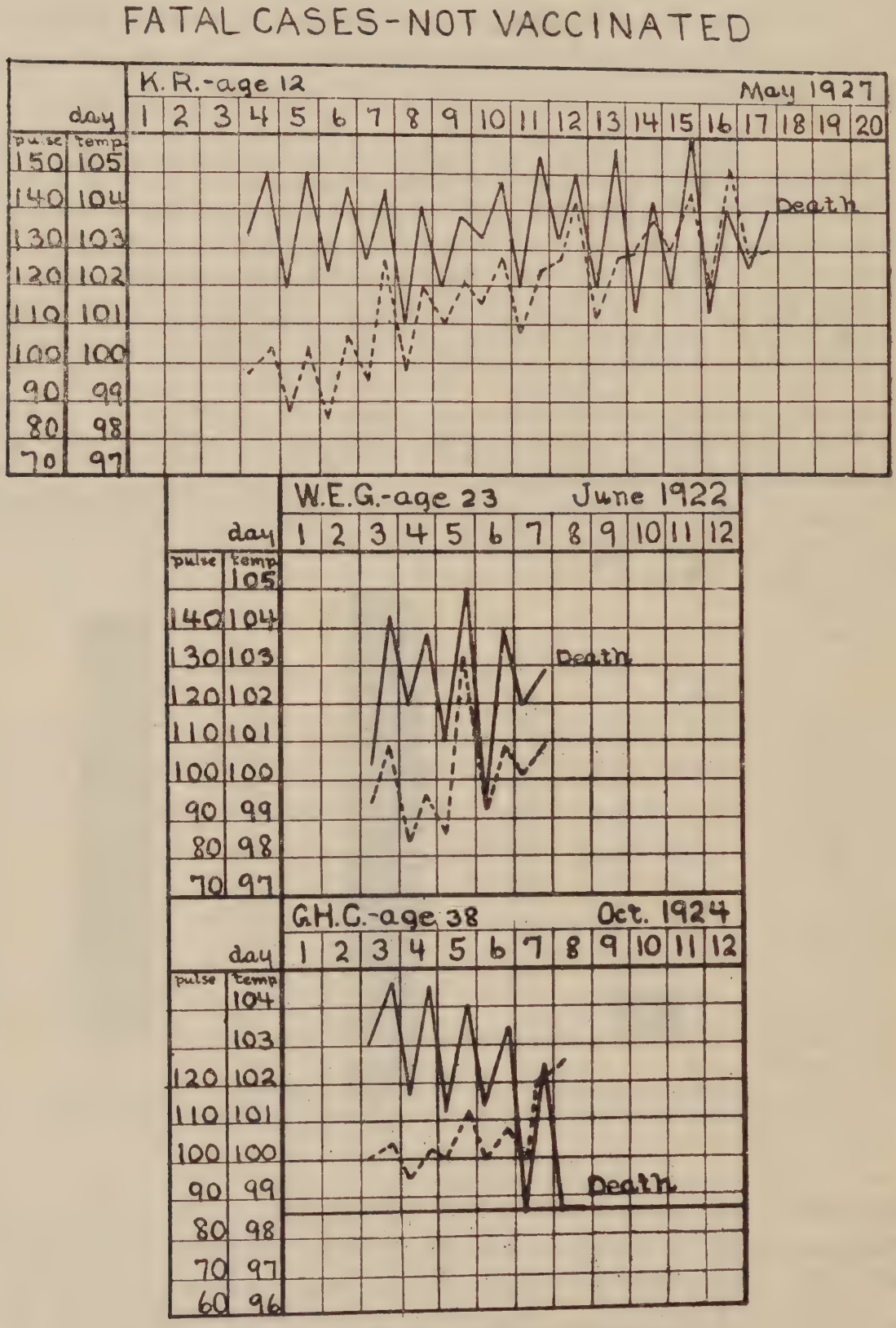

FIGURE 14.—Fatal cases—not vaccinated

years of age, were confined to the hospital or to bed for 30 days or longer, whereas none of the vaccinated cases was hospitalized for so long a period; and some of them, had they not received special care as Government beneficiaries, would have been discharged sev-

eral days earlier. These charts further show prolonged periods of high temperature in the nonvaccinated persons, whereas in those vaccinated the temperature was lower and began to drop much sooner.

Southern Idaho Test

The Southern Idaho test area is a small section of the north side of the Snake River valley and includes portions of Jerome, Gooding, Lincoln, Camas, and Blaine Counties. (Fig. 16.) On the south it is

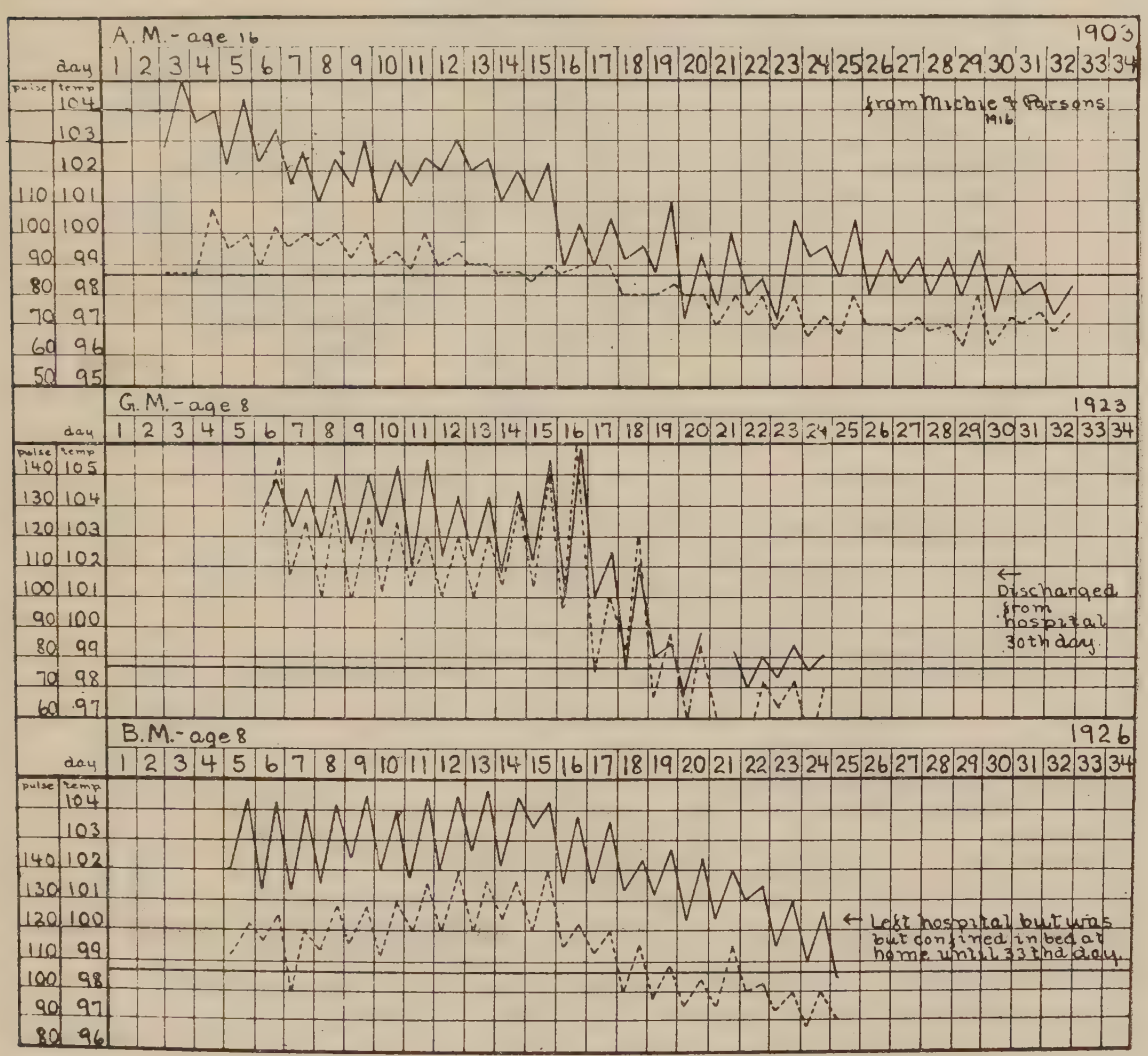

Figure 15.—Recovered cases—not vaccinated

bounded by the Snake River and on the north by the Sawtooth Mountains. The 60-mile expanse from river to mountains is mostly "sage-brush desert" country, especially in the northern portion. The farming communities, except in the southern part are mainly confined to small sections where irrigation water is available.

Spotted fever has been present for many years but there are no accurate year-by-year records of its prevalence. Such data as we have are as follows: 34 cases in 1910, 51 in 1911, 88 in 1913, 131 in 1915, 54 in 1917, 58 in 1918, 57 in 1919, 91 in 1920, 120 in 1921,

and 101 in 1923. These records are woefully incomplete, however, and for certain years represent half or less than half the test area.

Maxey in 1908 figured the southern Idaho mortality rate as 5.86 per cent. In the test area it was 10 per cent in 1926 (three deaths,

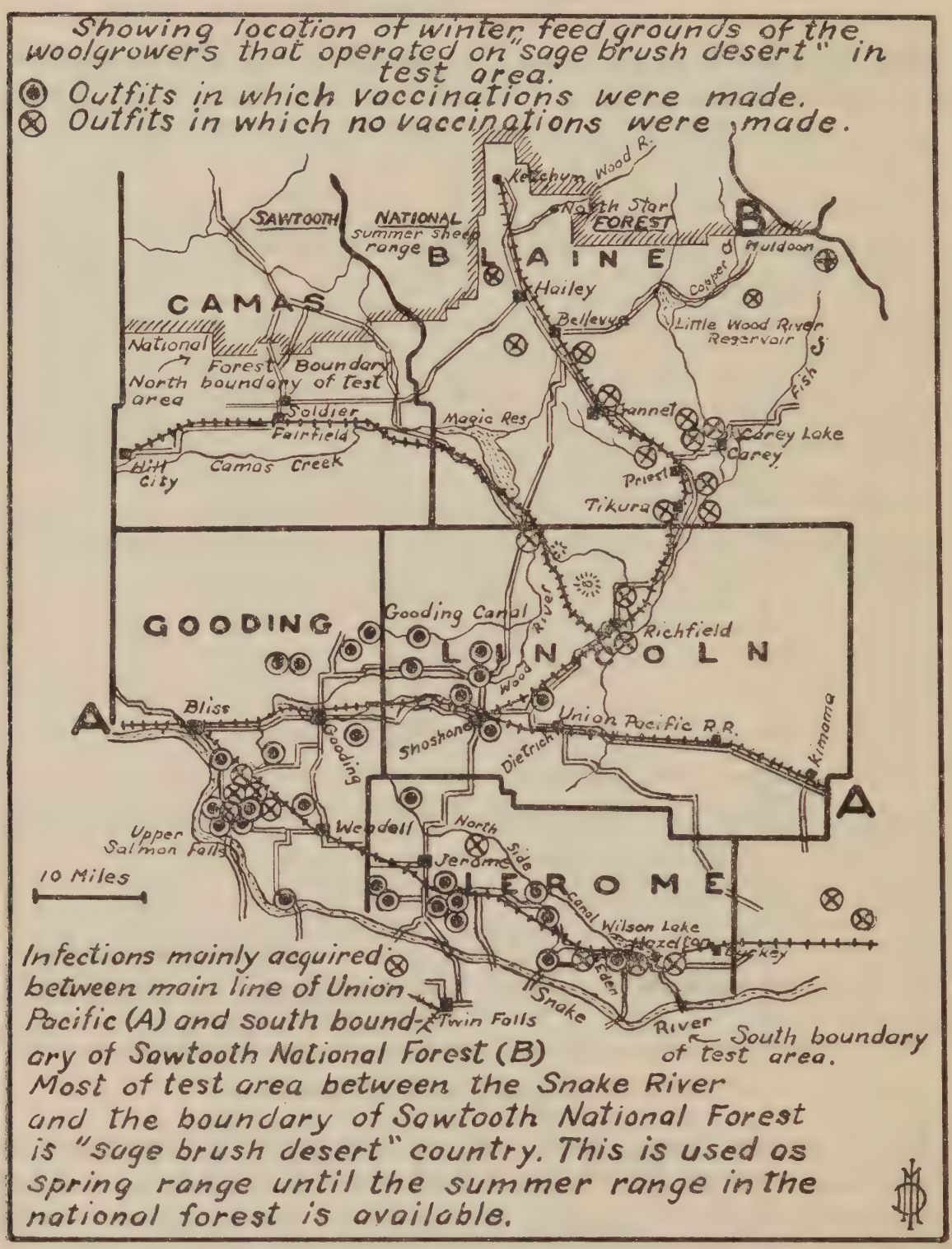

FIGURE 16.—Southern Idaho vaccination test area season of 1926

in persons, respectively, 45, 60, and 60 years of age) but there were no deaths in 1927.

There are even fewer data concerning the occupational distribution of cases. Maxey estimated that at least 38 per cent were among sheep herders. We believe that usually the percentage has been

greater. The next largest percentage is among those living on farms or engaged in allied pursuits. Cases also occur among sheep-shearers, miners and prospectors, section hands, fishermen, persons on picnics, and others who for various purposes go into areas where there is danger of tick bites.

Relatively few cases originate in Jerome County or in the more southern portions of Lincoln and Gooding Counties, most infections being contracted north of the main line of the Union Pacific Railroad. Certain areas have long been recognized as especially dangerous, but, as is true in other spotted fever districts, incidence in any particular section may vary markedly in different years.

As stated in the introductory remarks the relatively high percentage of cases that occur in persons handling sheep on the range (herders and camp tenders) made it feasible to limit our test to this one industry. Conditions within this industry that affect the test are therefore of interest.

During the winter months the sheep are held on winter feeding grounds in the irrigated sections, particularly in the southern portion of the area. As soon as the range grass has appeared in the spring, they are driven out on the " sagebrush desert " and gradually worked northward to summer ranges in the mountains. Since it is mainly in the " sagebrush desert " country (spring range) that cases of fever originate, the number that occur is materially influenced by the length of time spent on the " desert." This varies from year to year and is determined by seasonal factors, such as the time the spring feed appears on the range, the route followed, the amount of precipitation and the available water supply for the sheep. In the very dry seasons some owners ship their sheep to the mountains by rail, spending but little time in the sagebrush. The number of cases is also influenced by the meteorological conditions—the ticks, for example, being less active during dry springs—and by variations in tick abundance and in the prevalence of infection in ticks. Better working conditions imposed by State laws have in recent years favorably affected the spotted fever incidence in the sheep industry.

From an economic standpoint, spotted fever is a serious problem for the sheep owners. There have been seasons in which single outfits (some of the same in which we vaccinated) have had 8 or 10 of their men infected. Since infection takes place on the range, often many miles from the nearest physician, the problems of care, transportation to medical attention, and replacement are often difficult to meet. In earlier days when a band of sheep was often in charge of a single herder, infection of the latter and the resultant lack of attention to the sheep sometimes caused considerable losses.

Even now when the law requires that there be a herder and camp tender with each band, instances have occurred of both men becoming infected at the same time. It is not surprising, therefore, that we found a spirit of willing cooperation in most of the owners. This was particularly fortunate since the majority of the range men were Basques, many of whom spoke and understood English but imperfectly, and whose attitude toward vaccination was usually a reflection of that of their employer.

The vaccinations both seasons were made during the first two weeks in March when the herders were at or near the home ranches or winter feed grounds and before the sheep were started across the "sagebrush desert." When the test was initiated, it was hoped that half of the men in each outfit might be vaccinated, the other half serving as controls. This was found impossible if a sufficient number of herders were to be vaccinated to make the test of value. In some outfits all the men desired vaccination, in others the most urgent representations failed to secure a single man. As a matter of fact, however, subsequent knowledge of conditions showed the distribution of the vaccinated men in the various outfits to be of little significance, since, except in the smaller ones, the men of any one outfit did not necessarily traverse the same sections of the range and therefore were not subject to equal danger of infection. Even by vaccinating every man who desired it or who could be persuaded, it proved impossible to secure a half of the total range employees. Lack of success in some outfits was due either to prejudice or lack of interest on the part of the owners; but in many instances it was because it was necessary to make the vaccinations in the midst of the lambing season when many of the herders had to pitch hay a greater or less part of each day and consequently dreaded the possibility of sore arms. Most of the vaccinations were made in outfits that grazed their sheep in the western half of the area which is much more dangerous than the eastern portion.

When vaccinating, headquarters were established at Shoshone and daily trips of 70 to 130 miles were made into surrounding country; the men were vaccinated where chance found them—in sheep wagons, when loading hay, in feed corrals, etc. Under these conditions, an average of only 15 to 20 vaccinations a day could be made.

TEST DATA

The accompanying tables (27 and 28) give summaries of the data for the two seasons of the test.

In 1926 there were 63 woolgrowers with range sheep in the test area, each with from 1 to 20 range employees. The total number of herders and camp tenders was 373, of which 99 were spotted fever

immunes, leaving a test group of 274. Of this group 94 were vaccinated and 180 were left as controls. Eighteen of the vaccinated men received only one injection (2 c. c.). Among the controls there were, during the season, 13 cases of fever (1 in 13.85 men) and none among the vaccinated (0 in 94).

The outfits selected for vaccination were those in which the most cases had occurred in past years. This was because they operated in the most dangerous part of the test area. The results, therefore, appear even to better advantage when the data of the vaccinated (or most exposed) and nonvaccinated (or least exposed) outfits are segregated. In the 31 vaccinated outfits there were 203 employees with 71 immunes. Of the resultant test group of 132, 94 were vaccinated, leaving 38 controls. There was no case among the vaccinated men (0 in 94) and 5 (1 in 7.6) among the 38 controls. Three of these were single controls in outfits that had respectively 3, 4, and 5 nonimmunes. Of the other 2 cases, 1 was in an outfit that had 1 vaccinated man and 2 controls, the other in one that had 11 vaccinated men and 8 controls. Thirty-two outfits in which no vaccination was made employed 170 men with 28 immunes, leaving 142 controls. Of these, 8 became infected (1 in 17.75).

In 1927, the number of woolgrowers in the area was 58, of range employees 371, many of them new men since 1926. Eighty-eight were immunes, leaving 283 for the test. Of the latter 99 were vaccinated and there was a control group of 184. Of the 99 vaccinated men, 31 refused or were unable to take the second injection and it was not given to 31 others because of previous vaccination in 1926. The control group contained at least 14 men that had been vaccinated in 1926, but who refused vaccination in 1927. This number was doubtless larger but complete data could not be secured. Two injections of 2.5 c. c. (2 c. c. in 1926) each were used. One case of fever was reported in the vaccinated group (1 in 99). This was in a man (R. L. P.) 60 years of age, who had been vaccinated only in 1927 and would take but one injection. Among the controls there were 9 cases (1 in 20.45).

Again separating the data of the vaccinated and nonvaccinated outfits we have in the vaccinated subgroups 31 woolgrowers employing 211 men, of whom 48 were immunes, leaving 163 for test. Of these 99 were vaccinated and 64 were controls. As noted above, there was 1 case among the vaccinated men (1 in 99), and 4 among the controls (1 in 16). There were 27 outfits in which no men were vaccinated. These employed 160 men, of whom 40 were immunes. Of the 120 controls, 5 (1 in 24) contracted spotted fever.

Unfortunately the case of R. L. P., who was one of the vaccinated men, was not reported to us at the time of illness, and there was,

therefore, no opportunity to make clinical observations. The patient was infected near Hailey, in Blaine County, in an area from which cases are reported to be of more than average severity. After onset he made a trip of more than 100 miles to Rupert for treatment. He was hospitalized for 30 days. The patient's temperature, however, was not especially high, reaching only 102° F.

TABLE 25.—(*Southern Idaho Test, 1926*)

Number wool growers in test area	63
Number employees on range with sheep	373
Number immune employees	99
Number susceptible employees	274

TEST GROUP OF 274 SUSCEPTIBLES

Vaccinated	94	Not vaccinated	180
Spotted-fever cases	0	Spotted-fever cases	13 (1 in 13.85)

Five of the 13 cases among not vaccinated persons represented single cases occurring in each of five different outfits as follows:

Outfit No. 1, with 4 nonimmunes:		Outfit No. 3—Continued.	
Vaccinated	3	Not vaccinated	1
Cases	0	Cases	1
Not vaccinated	1	Outfit No. 4, with 3 nonimmunes:	
Cases	1	Vaccinated	1
Outfit No. 2, with 3 nonimmunes:		Cases	0
Vaccinated	2	Not vaccinated	2
Cases	0	Cases	1
Not vaccinated	1	Outfit No. 5, with 11 nonimmunes:	
Cases	1	Vaccinated	8
Outfit No. 3, with 5 nonimmunes:		Cases	0
Vaccinated	4	Not vaccinated	3
Cases	0	Cases	1

TABLE 26.—*Southern Idaho test, 1927*

Number of woolgrowers in test area	58
Number employees on range with sheep	371
Number immune employees	88
Number susceptible employees	283

TEST GROUP OF 283 SUSCEPTIBLES

Vaccinated	99	Not vaccinated	184
Cases	1 (1 in. 99)	Cases	9 (1 in 20.44)

Only meager data were obtainable from the attending physician. There were no heart or kidney complications and no delirium. The patient's advanced age (60 years) and his long trip after onset (requiring about two days) tended to aggravate his infection.

During the 2-year period of the test there were 557 nonimmune men on the range. Of these, 193 were vaccinated and 364 served as

controls. One case [23] of fever (in a man receiving but one injection of vaccine) occurred in the vaccinated men, 1 in 193, and 22 cases, 1 in every 16.55, among the controls.

OTHER VACCINATIONS IN SOUTHERN IDAHO TEST AREA

During the two years of the test, 143 persons outside the test group were vaccinated, 55 in 1926 and 88 in 1927. These included 30 sheep owners, 46 sheepshearers and others employed in shearing outfits, 39 farmers, members of farm households and persons employed on farms, 3 ditch riders, 6 predatory animal hunters, 1 Forest Service employee, 3 prospectors, 2 railroad employees, 2 wool buyers, 2 blacksmiths, and 9 others.

There were 36 cases of fever in the test area outside the test group, 17 in 1926, 19 in 1927. Of these, 27 were farmers, members of farm households, and persons employed on farms, 2 were wives of section foremen, 1 was a section hand, 1 a ditch rider, 1 a barber, 1 a sheepshearer, 1 a laborer on canal construction; and of the other 2, 1 had been picnicking, the other fishing. Three were fatal in 1926, none in 1927.

This record of vaccinations and case occurrence outside the test group bears but indirectly on the value of the vaccine, and is of interest because of the absence of cases among persons vaccinated.

DISCUSSION OF SOUTHERN IDAHO TEST

The above data are considered by us to suggest strongly that the vaccine in the dosage used will in most instances give full protection against the relatively mild type of infection dominant in southern Idaho, and perhaps even against the somewhat more severe type that occurs in other sections of the Northwest. Full protection may result from a single injection, since of the 81 men thus vaccinated (2 c. c. in 1926, 2.5 c. c. in 1927) but 1 became infected, whereas the incidence among the controls would point to the probability of five cases in the same number of unvaccinated men. Of this group, however, 31 who received a single injection in 1927 received two injections in 1926 and some residual immunity was doubtless carried over from the first season to the second; in fact, the one injection in 1927 was given because of this possibility.

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[23] During the two years several additional cases of spotted fever were reported in vaccinated men, but upon investigation their illnesses were found due to other causes. One had tularaemia, one pneumonia, and the others influenza. These cases had been diagnosed as "spotted fever without eruption"; a diagnosis which we feel is purely presumptive and rarely justified.

Use of Vaccine Outside of Test Areas

During the past three seasons, numerous requests for vaccine, far in excess of the supply, have been received from many points of the Northwest outside the two test areas. So far as possible vaccine has been supplied and the following vaccinations have been made: Montana 401, Idaho 203, Wyoming 863, Oregon 30, Nevada 5, Colorado 3, Utah 1; total 1,506.

Only two cases of spotted fever have been reported among the above-vaccinated persons. Both were patients of Dr. E. L. Jewell, of Shoshone, Wyo., and both became infected at the same sheep camp in the Kirby Creek district of infection southeast of Thermopolis, in Hot Springs County. The following brief-case records were forwarded by Doctor Jewell.

Case XIII

L. W., Thermopolis, Wyo., age 26, sheepherder.

Vaccination record.—1928: Received total of 3 c. c. of vaccine, 1 c. c. on each of following dates: April 5, 10 and 15.

May, 1928. "Was in bed only a week. Case started typically severe, with temperature of 102° to 104.5° F., lasting about three days, then gradually subsided for three days and was normal on sixth day. Continued so. Patient a little weak for four or five days."

Case XIV

G. L., Thermopolis, Wyo., age 17, "sheep business."

Vaccination record.—1928: Received 3 c. c. of of vaccine, 1 c. c. on each of following dates: February 22, 28, and March 13.

May, 1928. "Had a typical mild case and was in bed for three weeks, convalesced for a week, and is now at work about one month after onset."

Doctor Jewell further stated: "I had another case this spring from the same camp who was not vaccinated, who died promptly in six days, in spite of all I could do for him. I believe that all that have contracted spotted fever from this sheep camp have died except the above two vaccinated men. During the two preceding years four have died."

The Kirby Creek district in which these two cases originated is one of the few sections outside the Bitter Root Valley in which the death rate from Rocky Mountain spotted fever is extremely high. Except that it has been known to us for several years as an area of a severe type of infection we have no definite information of the mortality rate except that transmitted to us by Doctor Jewell and which has been verified from our station records.

The evidence at hand indicates that the results of vaccination in the above two cases are comparable with those against the virulent Bitter Root Valley strains.

Virus Neutralizing Properties of the Sera of Vaccinated Persons When Tested Against Bitter-Root Strains of Spotted Fever

Several tests (other than that recorded on p. 34) have been made which have demonstrated that the blood serum of a vaccinated person has definite virus neutralizing properties. One series of these tests, made February 2, 1927, will be given in detail and in stating results reference will be made to previous tests of the serum if the person concerned has been tested more than once. The sera of five vaccinated persons were used for the test, while those of two spotted-fever-recovered cases and one normal serum served as controls. For test purposes, constant amounts (0.5 c. c.) of each serum were mixed with graded amounts of serum virus from a guinea pig, and the resultant mixture, after standing one-half hour, was inoculated intraperitoneally into guinea pigs. The same serum virus collected from a single guinea pig was used in each instance. This was of the Robertson strain, the most virulent of several that were being maintained at the time of the test. The results, together with guinea-pig temperature charts, are graphically shown in Figure 17.

NORMAL SERUM CONTROL

H. H. Two guinea pigs received 1 c. c., two 0.25 c. c., and two 0.5 c. c. of serum virus.

Results.—No protective value. Five of the 6 guinea pigs died, 1 in 9, 1 in 11, and 3 in 12 days: 1 recovered. The incubation periods were, respectively, 6, 6, 6, 7, 6, and 5 days. All showed definite, typical, scrotal lesions.

SERA OF SPOTTED-FEVER-RECOVERED CASES—CONTROLS

Each of two sera from spotted-fever-recovered laboratory cases were tested in three guinea pigs, which received, respectively, mixtures that contained 0.1, 0.25, and 0.5 c. c. of serum virus.

A. M. C. (Case II above.) Onset August 13, 1926, 173 days before test. All guinea pigs were fully protected.

M. L. N. (Case III above.) Onset August 27, 1926, 159 days before test. These guinea pigs were but partially protected, the incubation periods being, respectively, 14, 11, and 7 days. There was a definite febrile period, but no scrotal lesions. The early 2-day rise in temperature in the guinea pig receiving 0.1 c. c. of virus was possibly due to secondary infection.

TEST SERA OF VACCINATED PERSONS

R. R. P.—*Vaccination record.*—1925: January 8, 1 c. c.; January 15, 1 c. c.; April 8, 2.5 c. c.; April 25, 1 c. c. 1926: February 16, 1 c. c.; February 23, 1.5 c. c.

Test made 344 days after last injection of vaccine. Four guinea pigs received, respectively, mixtures containing 0.1, 0.25, 0.5, and 1 c. c. of serum virus.

Result.—Full protection against all amounts of virus.

F. J. O'D.—*Vaccination record.*—1925: January 8, 1 c. c.; January 15, 1 c. c.; April 8, 1 c. c.; April 23, 1 c. c. 1926: February 16, 1 c. c.; February 22, 1 c. c.

Test made 345 days after last injection of vaccine.

Three guinea pigs received, respectively, mixtures containing 0.1, 0.25, and 0.5 c. c. of serum virus.

Results.—The guinea pigs receiving 0.25 and 0.5 c. c. of virus were fully protected. The one receiving 0.1 c. c. had a febrile period of four days beginning the fourteenth day after inoculation; this was likely due to intercurrent infection.

F. B. T.—*Vaccination record.*—1925: April 8, 2 c. c.; April 13, 2 c. c.; April 25, 1 c. c. 1926: February 16, 1 c. c.; February 22, 1 c. c.; August 28, 2 c. c.; September 3, 2 c. c.

Test made 155 days after last injection of vaccine.

Four guinea pigs received, respectively, mixtures containing 0.1, 0.25, 0.5, and 1 c. c. of serum virus.

Results.—The guinea pig that received 0.25 c. c. of virus was fully protected. Those receiving 0.1 and 0.5 each had mild febrile reactions, beginning, respectively, on the fourteenth and tenth day after inoculation, but it is not certain that the reactions were due to spotted fever. The one receiving 1 c. c. of serum (twice the amount of virus) had definite spotted fever, beginning the ninth day and terminating fatally on the twenty-first day.

R. R. S.—*Vaccination record and dates of tests.*—1924: May 19. first test; May 19, ½ c. c.; May 23, 1 c. c.; June 3, second test; June 17, 1 c. c.; June 21 1 c. c.; October 4, 1 c. c.; November 19, third test. 1926: February 10, 1 c. c.; September 28, 2 c. c.; October 7, fourth test. 1927: February 2, fifth test.

First and second tests.—The first test was made before any vaccine had been injected; the second 14 days after the second injection. Three guinea pigs were used for each test and each received a mixture containing 1 c. c. of serum and 1 c. c. of guinea pig virus. There was no protection. Had graded amounts of virus been used the second test might have shown some protection against the smaller volumes. Since this was not done, however, the above tests are not comparable with those which follow, except that in the fourth test, October 7, 1926, the guinea pig receiving equal parts of serum and virus was completely protected, suggesting an increase in virus neutralizing properties due to the subsequent vaccinations in February and September, 1926.

Third test.—This was made November 19, 1924, two further injections of vaccine having been received. Three guinea pigs each received 0.5 c. c. of serum and, respectively, 0.2, 0.3, and 0.5 c. c. of serum virus. Three control pigs were similarly inoculated with mixtures of normal serum and virus.

Results.—The guinea pig receiving 0.2 c. c. of virus was fully protected, while those receiving 0.3 and 0.5 c. c. had definite spotted fever. The former recovered and was later killed because of secondary infection, the latter died the twelfth day. All three control animals died, those receiving 0.2 and 0.3 c. c. virus on the eleventh day, the one receiving 0.5 c. c. on the tenth.

Fourth test.—During 1925 no vaccine was taken because of illness. In 1926 there were 2 injections, 1 in February and 1 in September. The fourth test was made October 7, nine days after the second injection. Three guinea pigs each received 0.5 c. c. of serum and, respectively, 0.1, 0.25, and 0.5 c. c. of serum virus.

Results.—All three animals were fully protected. This test shows a marked increase in the neutralizing power of the serum since the second and third tests made, respectively, June 3, and November 19, 1924.

Fifth test.—(The only one recorded in Figure 17.) This test made February 2, 1927, no additional vaccine having been received since the fourth test, 118 days before, except that a fourth guinea pig was used which received 1 c. c. of virus. Procedure was the same.

Results.—The 0.1 c. c. Guinea pig was fully protected, while those receiving the larger amounts of virus all became infected. The 0.25 and 1 c. c. animals died, respectively, on the twentieth and nineteenth days with definite scrotal lesions, despite prolonged incubation periods, respectively, 12 and 11 days. The 0.5 c. c. animal ran a low fever beginning the fourteenth day, but scrotal lesions were absent and recovery was complete.

E. W. M.—*Vaccination record and dates of tests.*—1924: November 24, 1 c. c.; December 12, 1 c. c.; December 15, 1 c. c.; December 27, first test. 1925: April 25, 1 c. c. 1926; February 16, 1 c. c.; February 22, 1 c. c. 1927; February 2, second test.

First test.—This was December 27, 1924, 12 days after the third injection of vaccine. Three guinea pigs each received 0.4 c. c. of serum and, respectively, 0.1, 0.2, and 0.3 c. c. of serum virus. Three controls received identical amounts of virus.

Results.—The three control animals died, respectively, in 8, 7, and 9 days, the test guinea pigs in 12, 13, and 10 days. The latter showed longer incubation periods and lower fever. It is impossible to state whether the prolonged courses of infection were due to a nonspecific action of the serum or to a virus neutralizing property. The latter, if present, was slight.

Second test.—Made February 2, 1927, 345 days after last injection of vaccine. Three guinea pigs each received 0.5 c. c. of serum and, respectively 0.1, 0.25, and 0.5 c. c. of serum virus.

Results.—The guinea pig receiving 0.25 c. c. of virus was fully protected. The two others both developed definite spotted fever with typical scrotal lesions, dying, respectively, on the fifteenth and nineteenth days. This test shows increased neutralizing power of the serum as compared with the first test.

DISCUSSION

The results of the normal human serum and spotted-fever recovered serum control tests accord with our experience in other similar experiments. Of six guinea pigs inoculated with normal serum-guinea pig virus mixtures, five died, although as frequently happens in such tests the incubation period was slightly prolonged (it is normally three or four days). Of the two tests with sera of persons recovered from spotted fever, made, respectively, 173 and 159 days after onset, the former resulted in full protection while in the latter none of the three guinea pigs was fully protected, but there were definitely prolonged incubation periods, an absence of scrotal lesions and all recovered. These results agree with previous observations of the virus neutralizing properties of the sera of recovered cases; those from especially mild cases seldom affording more than partial protection.

Of the five vaccinated persons whose sera was similarly tested, all had received at least six injections of vaccine in the previous

3-year period. For three of these, R. R. S., R. R. P., and E. W. M., it is known that prior to the first injection of vaccine, their sera had no protective value when mixed with an equal volume of virus. By referring to Figure 17, it may be seen that the technic of tests was identical with the control tests of normal serum except that of the sera of R. R. P., F. B. T., and R. R. S., an additional mixture containing twice as much virus (1 c. c.) as human serum was inoculated. The serum of R. R. P. afforded complete protection even to the guinea pig receiving the double volume of virus, while those of F. J. O'D. and F. B. T. gave complete or almost full protection against the 0.1, 0.25, and 0.5 volumes of virus. That of F. B. T., however, did not prevent typical fever and death in the guinea pig that received 1 c. c. of virus. The sera of R. R. S. and E. W. M. show less neutralizing value than those just noted, only one guinea pig of each test group being fully protected, and two of each series dying with the usual scrotal lesions. It is of interest to note that the serum of R. R. S., which afforded but little protection in the present test, gave full protection when similarly tested 109 days earlier on October 7, 1926, nine days after his last previous injection of vaccine.

Of the 18 guinea pigs used in these five tests, 9 were fully protected. In 5 of the 9 in which infection was not prevented the incubation periods were markedly prolonged, the febrile course short and mild, scrotal lesions absent, and recovery followed. Of the 4 that died, 3 had long incubation periods and death did not occur until the nineteenth and twentieth days.

We interpret these results as showing definitely that the vaccine has provoked a virus-inhibiting response in the vaccinated persons, but we realize that the test results can not be taken as an index of relative individual protection. This limitation is amply indicated by the tests with the sera of the two recovered cases, A. M. C. and M. L. N., both of whom were undoubtedly fully immune, even though the serum of the latter afforded but partial protection to guinea pigs.

Reactions

Reactions to the vaccine have mostly been inconsequential and limited usually to local redness, swelling, and itching about the site of infection. The swelling sometimes extends below the elbow and occasionally to the hand. Itching has been the most common complaint. This local reaction either disappears or becomes scarcely noticeable in persons who have been vaccinated several times. Less than 5 per cent of 3,893 vaccinated persons have complained of constitutional symptoms. These have included malaise, slight fever,

nausea, and aching joints or muscles. Protein reactions with urticaria and oedema have occurred in about 1 per cent of the persons vaccinated, the symptoms usually appearing very shortly or within a few hours after injection of the vaccine, but in one instance were

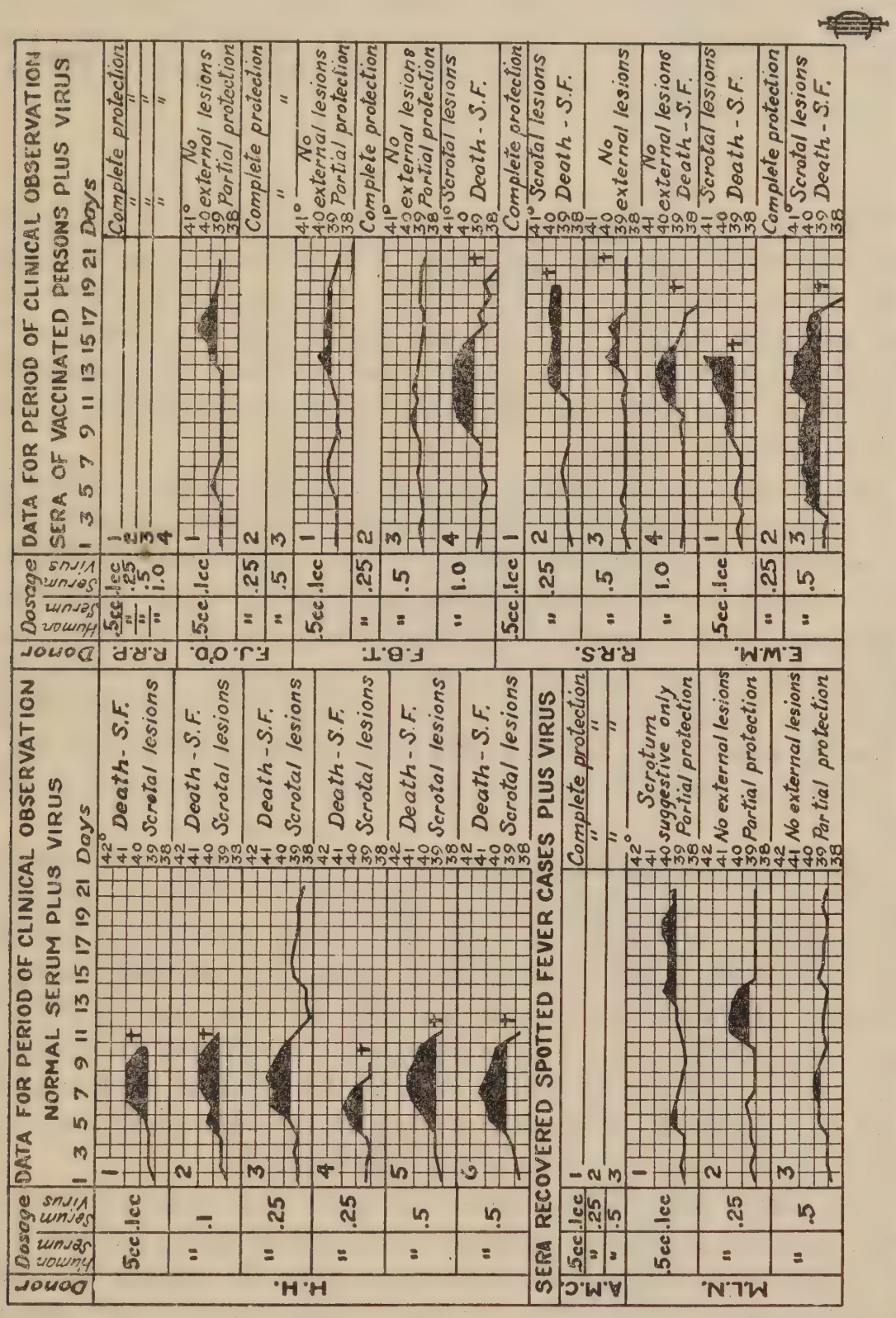


FIGURE 17.—Temperature charts of guinea pigs showing virus neutralizing properties of sera of vaccinated persons

delayed 10 days. The symptoms last from a few hours to several days, but in one man the urticarial rash was more or less persistent over a period of nine months, finally disappearing following infection with Rocky Mountain spotted fever. (See Case I.) Of three

of the persons who experienced this reaction following the initial injection and returned for the second, two felt no discomfort after the latter, which was given at the regular 5-day interval, but in the third the symptoms were repeated and somewhat aggravated. One person, who had had only a local reaction when first vaccinated, had a definite urticarial rash when again vaccinated the next year. Marked collapse occurred in one person who, the next day, felt normal except for weakness.

SUMMARY

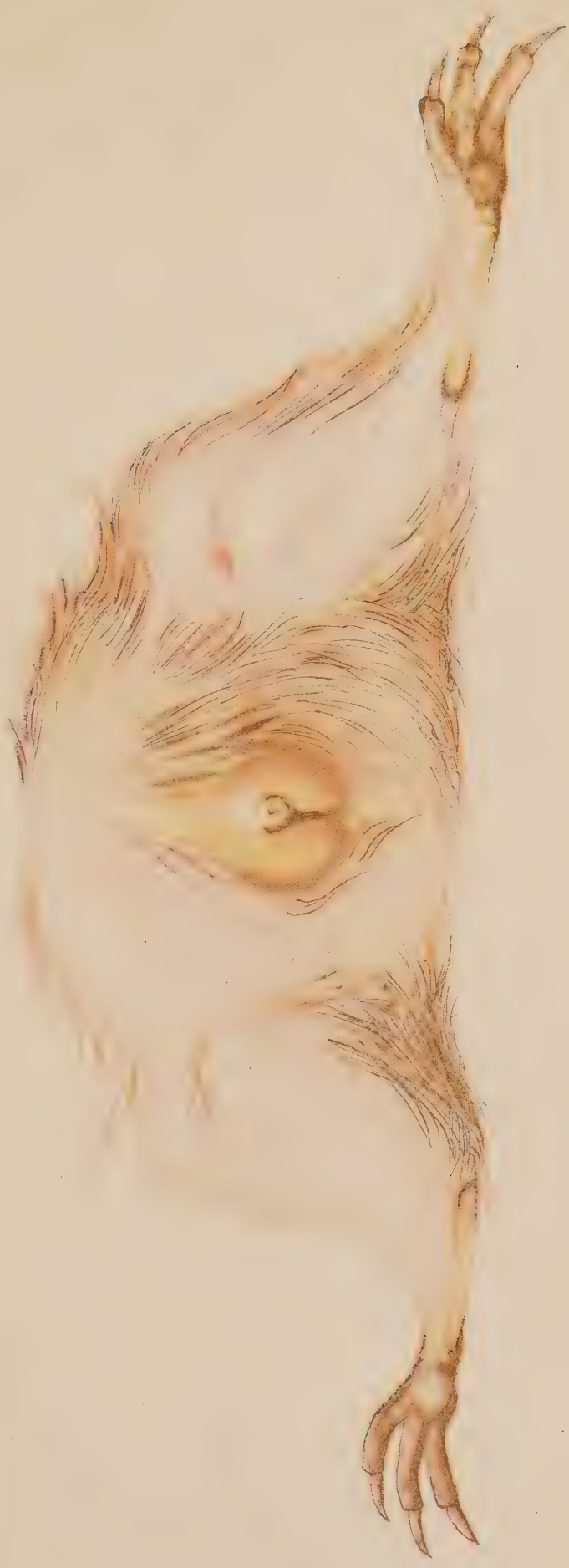
1. Tests have been made in local sections of Montana and Idaho to determine the value of phenolized tick virus as a prophylactic against Rocky Mountain spotted fever. The vaccine has also been distributed upon request to physicians in other infected areas of the Rocky Mountain region. The dosage used has been purely arbitrary and for adults has most commonly been two injections of 2 c. c. each, at 5-day intervals.

2. The Montana test, against the *most virulent* type of infection, has been made in the Bitterroot Valley during the 4-year period, 1925 to 1928. The mortality among nonvaccinated adult cases was 90.91 per cent (10 of 11 cases) but among those vaccinated was only 9.09 per cent (1 of 11 cases). The mortality rate for nonvaccinated adults for the 12-year period, 1917 to 1928, was 84.91 per cent. The mortality among laboratory workers exposed to infection with Bitterroot Valley strains was 100 per cent (5 of 5 cases) before the use of the vacine, but has subsequently been only 14.28 per cent (1 of 7 cases).

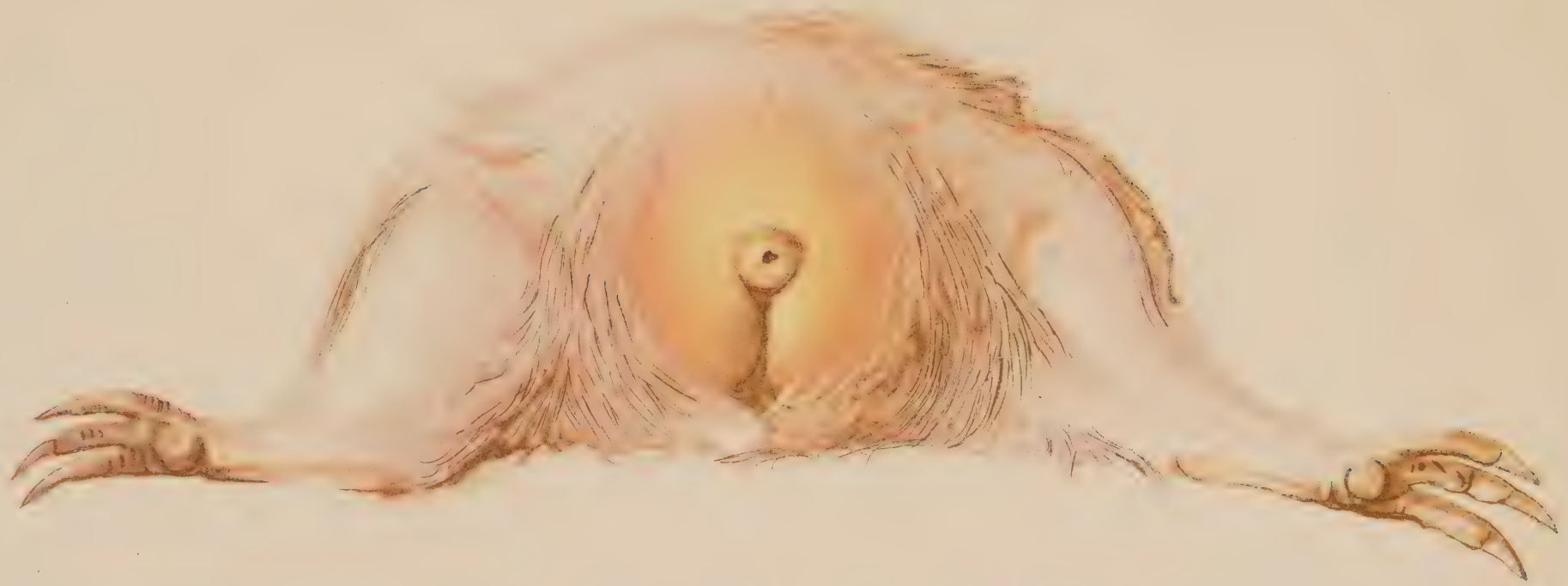
3. The Idaho test against the *mildest* recognized type of infection, was made in a small section of the Snake River Valley of southern Idaho, during the seasons of 1926 and 1927. Because of occupational incidence this test was confined to persons handling sheep on the range. During the two seasons 1 case occurred among 193 vaccinated men and 22 cases, 1 in every 16.55 men, among 364 controls. If only men in the sheep outfits exposed to greatest danger of infection be considered, there was 1 case in 168 vaccinated men and 13 cases, or 1 in every 9.3 men, among 121 controls.

4. The Bitter Root Valley data definitely show that partial protection, sufficient to insure relatively mild infections and recovery, is usually conferred against the extremely virulent strains of Rocky Mountain spotted fever. The southern Idaho data strongly suggest that full protection is commonly given against the milder type of infection which is prevalent in most sections of that region.

5. Two additional cases have occurred among 1,506 persons vaccinated in other sections of the Rocky Mountain region. These were



Normal Guinea Pig



A.Hoen & Co Baltimore

Spotted Fever in Guinea Pig showing redness and swelling of testicle
on 7th day after inoculation

PLATE 11

A. Hoen & Co. Baltimore.

Spotted Fever in Guinea Pig showing gangrenous condition of testicle
on 10th day after inoculation

PLATE 12

from a focus of virulent infection on Kirby Creek in Wyoming. Both recovered, and were the only recoveries of seven cases from that focus in the past three years. The results were apparently comparable with those against the similar highly virulent strains in the Bitter Root Valley.

6. The blood serum of vaccinated persons shows definite virus neutralizing properties.

7. Reactions to the vaccine are no severer than those following the subcutaneous injection of other biological products.

8. The vaccine has a definite value as a prophylactic against Rocky Mountain spotted fever.

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In connection with the Bitter Root Valley test the writers desire to express their appreciation to the local physicians who, without fee, have public-spiritedly assisted in the administration of the vaccine. They are particularly indebted to those physicians who have attended the vaccinated cases for their deep interest and cooperation, and especially to Dr. Herbert Hayward, of Hamilton, who attended 10 of the 12 local cases in vaccinated persons and whose considerable experience with the local type of infection during 17 years residence in the Bitter Root Valley has been of great value to us in observing and comparing the clinical features of his cases. We further gratefully acknowledge his assistance in preparing the records of his cases.

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During the season of 1926, due to the illness of one of us (Spencer), Passed Asst. Surg. L. B. Byington was temporarily assigned to the vaccination studies particularly for the purpose of following through the Idaho test. He also aided in the work in the Bitter Root Valley tests during periods when his presence in Idaho was not essential. His assistance materially contributed to the satisfactory carrying out of the season's work in both areas.

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